Comparison of Nutritional Value Between Kefir Milk (KM) and Fresh Milk (FM) in Goat Milk

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DECLARATION

I hereby declare that the work embodied in here is the result of my own research except for the excerpt as cited in the references.

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Comparison of Nutritional Value between Kefir Milk (KM) and Fresh Milk (FM) in Goat Milk

ABSTRACT

Kefir is known worldwide for its advantages which are most important to lactose intolerance symptoms associated with good probiotic bacteria and yeast. However, in Malaysia there is a lack of awareness in kefir product. Kefir can be derived from dairy animals such as cow, buffalo and goat. Nevertheless, reports on kefir derived from goat are scarce. Thus, the objectives of this study are (1) to compare the nutritional value between kefir milk (KM) and fresh milk (FM) in goat milk and (2) to compare the effect of storage time between (KM) and (FM) in goat milk. Therefore, this study will start with collection of raw goat milks and mixing of kefir grains in raw goat for (KM) sample only. After that, the sample will transport to undergoes milko scan machine for scanning the nutritional level of milk; fat, protein and lactose at Veterinary Faculty of University Malaysia Kelantan (UMK). Then, the sample have triplicated in Day 1, 4 and 8. The results indicated that the percentage of lactose in (KM) was significantly decreases from Day 1 to Day 8, but in (FM), in Day 4 was slightly decreases from Day 1 but increasing in Day 8. The percentage of fat in (KM) was significantly increase from Day 1 to Day 8, but in (FM) the value of mean in Day 8 was decrease from Day 4; 4.81 to Day 8; 3.97. Next, the percentage of protein in (KM) was significantly increases from Day 1 to Day 8 but in (FM) the percentage of protein was increases from Day 1 to Day 4 but slightly decreases in Day 8. In addition, the nutritional value between (KM) and (FM) samples were not significant ($p>0.05$) when the p-value of lactose, fat and protein were $p=0.47$, $p=0.76$ and $p=0.53$ respectively in Day 1. In Day 4, all the sample were significant (lactose, $p=0.0001$, protein, $p=0.04$ but not in fat content, $p=0.27$. However, in Day 8, all the sample were significant value, ($p<0.05$). It shows that all the sample in Day 8 were significantly difference; ($p<0.05$). In conclusion, it is seen that (KM) derived from goat milk produces the higher nutritional value in protein and fat content than (FM) but lower in lactose level.

Keywords: Lactose intolerance, Lactic acid bacteria, Kefir grain

Perbandingan nilai pemakanan di antara susu kefir (KM) dan susu segar (FM) di dalam susu kambing

ABSTRAK

Kefir dikenali di seluruh dunia kerana kelebihannya yang penting untuk individu yang tidak dapat meminum susu dan juga berkaitan dengan bakteria probiotik yang baik dan yis. Walau bagaimanapun, di Malaysia terdapat kekurangan kesedaran dalam produk kefir. Kefir boleh diperolehi daripada haiwan tenusu seperti lembu, kerbau dan kambing. Walau bagaimanapun, laporan mengenai kefir diperolehi daripada kambing adalah terhad. Oleh itu, objektif kajian ini adalah (1) untuk membandingkan nilai pemakanan di antara kefir susu (KM) dan susu segar (FM) dalam susu kambing dan (2) untuk membandingkan kesan masa penyimpanan antara (KM) dan (FM) dalam susu kambing. Oleh itu, kajian ini akan bermula dengan pengumpulan susu kambing mentah dan mencampurkan kefir bijirin dalam kambing mentah untuk sampel (KM) sahaja. Selepas itu, sampel akan dihantar ke Mesin 'Milko scan' untuk mengimbas tahap pemakanan susu; lemak, protein dan laktosa di Fakulti Veterinar Universiti Malaysia Kelantan (UMK). Kemudian, sampel telah diulang tiga kali pada hari 1, 4 dan 8. Hasil kajian menunjukkan bahawa peratusan laktosa dalam (KM) telah berkurangan daripada hari pertama ke hari 8, tetapi dalam (FM), dalam hari 4 telah berkurangan sedikit dari hari 1 tetapi meningkat dalam hari 8. Peratusan lemak (KM) telah meningkat dengan ketara daripada 1 hari ke hari 8, tetapi dalam (FM) nilai min dalam hari 8 telah berkurangan dari hari 4; 4.81 hingga hari 8; 3.97. seterusnya, peratusan protein dalam (KM) telah meningkat dengan ketara daripada 1 hari ke hari 8 tetapi dalam (FM) peratusan protein telah meningkat dari hari ke-4, tetapi penurunan sedikit dalam hari 8. Di samping itu, nilai pemakanan di antara (KM) dan (FM) sampel tidak ketara ($p > 0.05$) apabila nilai p-laktosa, lemak dan protein adalah $p = 0.47$, $p = 0.76$ dan $p = 0.53$ masingmasing dalam hari 1. Dalam hari 4, Semua sampel adalah penting (laktosa, $p = 0.0001$, protein, $p = 0.04$ tetapi bukan dalam kandungan lemak, $p = 0.27$. Walau bagaimanapun, pada hari 8, Semua sampel adalah nilai yang ketara, ($p < 0.05$). Ia menunjukkan bahawa semua sampel dalam hari 8 adalah perbezaan yang ketara; ($p < 0.05$). Kesimpulannya, ia dilihat bahawa (KM) berasal daripada susu kambing menghasilkan nilai pemakanan yang lebih tinggi dalam kandungan protein dan lemak daripada (FM) tetapi lebih rendah dalam tahap laktosa.

Kata kunci: laktosa sikap tidak bertoleransi, bakteria asid laktik, Kefir bijirin

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LIST OF ABBREVIATIONS AND SYMBOLS

(KM)	Kefir Milk
(FM)	Fresh Milk
(LAB)	Lactic acid bacteria
PUFA	Polyunsaturated fatty acid
DNA	Deoxyribonucleic acid
Ca	Calcium
P	Phosphate
SEM	Standard Error Mean
ANOVA	Analysis of Variance
LI	Lactose Intolerance
EFAs	Essential Fatty Acid
CLAs	Conjugated Linoleic Acid
MalayKef	Malaysia Kefir
UniSZA	Universiti Sultan Mizan Zainal Abidin
^{0}C	Degree Celcius

CHAPTER 1

INTRODUCTION

1.1 Research Background

Kefir is the best combination of complex mixture and specific milk production between bacteria and yeast. The process of fermentation of dairy product that originate from Caucasus Mountains (Atalar *et al.*, 2015). The history of word "kefir" derived from Turkish word '*keif*' which means 'well feeling' (Machado *et al.*, 2013 ; John and Deeseenthum, 2015; Cais-Sokolińska *et al.*, 2015) and. Kefir consist of non-pathogenic bacteria, especially *Lactobacillus sp*. (Wang *et al.*, 2017). Most of kefir production differs from other fermented products because from kefir grains. Kefir grains consist of complex mixture and specific lactic acid and acetic acid that generate the symbiotic bacteria (Reis *et al.*, 2017). Fiorda *et al.*, (2017) also reported that kefir grain derived from a complex microbial associated among yeast and bacteria, thus as a starter culture for milk fermentation process. Kefir production is divided into two categories; traditional and industrial methods (Atalar *et al.*, 2015).

The main difference between two techniques is the inoculation of kefir grain or culture into all types of milk (Atalar *et al.*, 2015). For traditional method, kefir grains are commonly use and this is the easiest way to culture the milk kefir than modern method (Reis *et al.*, 2017). In addition, according to the most variability microflora in kefir grains, the results of kefir sample shows promising differences way regarding on origin of the grains and condition while handling and storage (Atalar *et al.*, 2015; Reis *et al.*, 2017). For standard production, the kefir

starter cultures were used in many industrial brands that composed from pure kefir microflora strains (Atalar *et al.*, 2015). The traditional kefir production commence when milk undergoes fermentation process with a starter culture of small, round-shape and gelatinous grains (Machado *et al.*, 2013). The taste of milk kefir is slightly smooth, creamy, bubbly texture and tartness (Reis *et al.*, 2017). On the other hand, John and Deeseenthum (2015) was reported that several method is prepared by carrying out traditional kefir fermentation to produce a kefir-like beverage.

Currently, milk kefir has been comprising and most admirable by connoisseurs of fermentation due to numerous beneficial of health (Reis *et al.*, 2017). John and Deeseenthum (2015) also reported milk kefir have more advantages such as immunological, antibacterial and antitumor for consumer. On the other hand, this is promising for active cancer survivors because milk kefir production; good source of protein can inhibit cell cancer from others background of cancer (John and Deeseenthum, 2015). However, in recent studies, the role of probiotics such as lactic acid bacteria (LAB) is getting more vital for dangerous diseases such as rheumatoid arthritis and cancer (Bourrie, Willing and Cotter, 2016; Sharifi *et al.*, 2017). In addition, kefir also help in improvement of physiochemical for active cancer survivors during and after treatment (Boeneke *et al.*, 2017). Besides, kefir also can be produced from whole, semi-skimmed or skimmed pasteurized of goat, cow sheep or buffalo milk (John and Deeseenthum, 2015; Reis *et al.*, 2017). Hence, this study will be carried out to analyses of milk kefir and fresh milk derived from goat milks with their actual composition of physiochemical in milks including benefits.

1.2 Research Problem Statement

In this modern area, many supplement or medicines had been developed to improve the health of communities. This also to protect the citizens from various of killer disease such as cancer, heart attack, contagious disease and more. The prevalent disease also normally comes from our habit and behaviour in daily life. Besides, medical practitioner and researcher are putting many efforts to solve this problem regarding several diseases that dispreads continuously year by year. In addition, influx of foreigner also can spread new diseases from their origin to our population.

There had been numerous reports elucidating health benefits from kefir derived from cow and buffalo milk (Atalar *et al.*, 2015; Wang *et al.*, 2017; Zalewska, Kaevska and Slana, 2018). However, recently reports regarding kefir derived from goat milk is scarce. Thus, it is important to compare milk quality in kefir from goat and cow milk and its various beneficial value to consumer.

1.3 Research Objectives

1. To determine the nutritional value in kefir milk and fresh milk derived from goat milk.
2. To compare the effect of storage time in kefir milk and fresh milk derived from goat milk

1.4 Research Hypothesis

The study comprises of two hypotheses:

H_o: The nutritional value is similar in kefir milk and fresh milk derived from goat milk.
H_1: The nutritional value is different in kefir milk and fresh milk derived from goat milk.

H_o: The effect of storage time is similar in kefir milk and fresh milk derived from goat milk.
H_1: The effect of storage time is different in kefir milk and fresh milk derived from goat milk.

1.5 Research Scope

This study will be carried out by using raw milk from goat. Next, milk sample will be divided into fresh milk and kefir milk. Each bottle will be filled with 250ml of goat milk and for kefir milk samples were add up with 5ml of kefir grains. Then, the milk samples from fresh milk were incubated in freezer below 4ºC and kefir milk samples will incubated in laboratory racks in room temperature for probiotic reaction with kefir grain and milk sample. The samples will be carry out in Day 1, Day 4 and Day 8 to be scan using Milko scan for nutritional value determination (fat, protein and lactose). All the data were recorded in Excel Word sheets.

1.6 Significance of Study

Kefir contain various of health benefits such as reducing cholesterol level, antitumor and improving lactose tolerance. This promising milk product also good for pregnant and cancer survivor in order to increase immunity. Kefir can make communities life more lasting instead of pioneer probiotic culture. The researchers also already proven about 250 cups of store-bought yogurt is equal to 1 cup of homemade kefir. Kefir also can be made from any dairy milk but will resulting various level of physiochemical proportion benefits in the milks. Thus, this study was designed to analyse and compare the nutritional value in fresh milk and kefir milk derived from goat milk

CHAPTER 2

LITERATURE REVIEW

2.1 Kefir

Kefir is a fermented drink beverage which is consist of various types probiotic bacteria associated with yeast in symbiotic matrix. The name "kefir" is supposedly derived from Turkish word "keyif" means "cheerful feeling" (Nielsen, Gürakan and Ünlü, 2014). Machado *et al.*, (2013) has reported that kefir consumption is rising in many countries year by year especially in Southern Asia and Eastern Europe regarding to its exclusive tastes and good various effect on human health.

Kefir is reflected as good microbiologically and chemically due to their acidic purpose and ability to prevent the survival of pathogenic organisms to consumer (Zalewska, Kaevska and Slana, 2018). Besides, it taste is normally creamer, smoothly and sour, which can easily digested (Sharifi *et al.*, 2017). Traditionally, many of kefir products will use dairy milk products such as cow, goat and buffalo but in certain country which have expensive value of dairy milk or minimally consumed, they normally used food product likes soy milk and coconut milk as kefir production (Prado *et al.*, 2015). Recently, kefir is enhancing scientific community to do further researches due to its numerous beneficial effects of health (Reis *et al.*, 2017).

Moreover, kefir also have abundant of good potential related with goat milk. Harmayani, (2016) had reported kefir which associated with goat milk can prevent the community of development countries with higher cases for all types diabetes mellitus from suffered this disease. Besides, it also can increase antioxidant activities especially for food storage product and vital for increase in the proportion of polyunsaturated fatty acids (PUFA) resulting numerous benefit of health (Cais-Sokolińska *et al.*, 2015; Yilmaz-Ersan *et al.*, 2015).

2.2 History of Kefir

Sharifi *et al.*, (2017) reported the fermented drink beverage of kefir originates from Caucasian and Tibet. Then, kefir had originated in the Caucasus, Tibetan and Mongolian mountain (Reis *et al.*, 2017). Besides, Prado *et al.*, (2015) also reported the first origins of kefir were originated in the Balkans, in Eastern Europe, and in the Caucasus. In addition, (Hernández-Ledesma *et al.*, 2010; Akbağ *et al.*, 2011) were also reported the same origins of kefir is located at Caucasus mountain. Moreover, the most excellent probiotic beverage, kefir also comes from the Caucasus mountain (Machado *et al.*, 2013). After that, (John and Deeseenthum, 2015; Rafie *et al.*, 2015) were also reported that the power of fermented milk drink, kefir originated in the Caucasus Mountains of Russia.

2.3 Benefits in health

2.3.1 Cancer treatment/survivor

Kefir has vital component of probiotic microorganism especially in treatment of cancer. This is because kefir is made up with numerous of good probiotic bacteria (König and Fröhlich, 2017). Kefir effectively inhibits leukemia cell proliferation and It had proapoptotic effects on normal cells in cancer cells without significant necrotic effects (Rafie *et al.*, 2015). Then, Reis *et al.*, (2017) reported composition of good probiotic bacteria in kefir such as (*Lactobacillus acidophilus spp, Lactococcus spp and Streptococci spp)* can improve human lactose digestion and lactose tolerance thus give totally prevention from lactose intolerance and maldigestion. Moreover, cancer survivor also can do their daily exercise as well as other healthy human because they just only consume kefir drink to improve recovery of the bodies immunity after exercise (Boeneke *et al.*, 2017). Furthermore, kefir also give an extra advantages cancer treatment such as decreased deoxyribonucleic acid (DNA) damaged, decreased tumour growth and increase immune response to consumers (Sharifi *et al.*, 2017). Mostly, researchers had successfully proved that role of kefir is important in various cancers instead antitumor process.

2.3.2 Osteoporosis patient

Dairy products are the main source of calcium (Ca), but the loss of the consumption habit contributes to low consumption in adulthood, which leads to osteoporosis and increased fracture risk. So, many researchers investigate the composition of kefir in different types of milk instead of its health benefits. According to Fina, Brun and Rigalli, (2016), it has been well documented that people in hypolactasia are better able to accept fermented milk such as kefir and (Ca) absorption in lactose-hydrolysed is higher than lactose-free milk. In addition, (Ca) supplementation from dairy product fermented with good probiotic bacteria such as kefir in dietary women over the age of 55 from Zabrze, Poland are higher than unfermented dairy product (Martela *et al.*, 2018). Moreover, kefir enhances bone mass, density and structure with calcium absorption that enhances bone structure and developmental cell bioactivities in human (Chen *et al.*, 2014).

2.4 Milk processing

2.4.1 Fermentation

Milk is an excellent human and bacterial food source. At room temperature, milk is naturally sourced from lactic acid produced from lactose fermentation. Fermentation is occurring when bacteria, yeast or other microorganisms takes place in chemical breakdown of a substance such as milk (Granier, Goulet and Hoarau, 2013). Milk fermentation is directly process from lactic acid with creamy and slightly acidic flavour depending numerous mixture

between selected bacteria and yeast (Castillo Martinez *et al.*, 2013). In addition, as a potential microorganism in the fermentation process, lactic acid bacteria are widely used in food fermentation throughout the world, especially in transforming milk into good quality fermented milk products (Widyastuti, and Febrisiantosa, 2014). Furthermore, fermentation is one of the ancient and most economical ways of preserving food in the world (Altay *et al.*, 2013).

2.5 Milk nutritional value

2.5.1 Goat milk

Goat milk is an important nutrient for humans, particularly those with lactose intolerance problems and sensitivity to the milk of other animals. Goat milk consumption and production has recently increased globally especially in developed nations recognize the advantage of goat milk. Goat milk is well known to have a high nutritional value compared to other animal species such as calcium (Ca): 1.2 gram and 1 g phosphate (P) per litre (G *et al.*, 2016). In addition, casein fraction also is higher in goat milk with 80% of milk protein (Claeys *et al.*, 2014). This fraction also makes goat milk very good material for raw processing, especially in cheese making (Barlowska *et al.*, 2011). Moreover, Sant'Ana *et al.*, (2013) also reported goat milk with high biological value and chemical composition with lower allergenic potential are very suitable in raw processing in cheese.

CHAPTER 3

METHODOLOGY

3.1 Location

Universiti Sultan Zainal Abidin (UniSZA) Tembila Campus, Pasir Akar farm in Besut Terengganu and UniSZA laboratory.

3.2 Apparatus and Equipment

The raw goat milk was supplemented at UniSZA farm respectively. Kefir grains were ordered from 'MalayKef 'in Facebook online respectively. The price of the grains for 20 grams was Rm 50.00. The kefir grain was only use in kefir milk sample with 5ml for each kefir milk bottles samples. All the bottle samples from 1st collection until 3rd collection was 250ml of raw goat milk. In addition, all the sampling preparation were doing at Pasir Akar farm, UniSZA Tembila Besut Campus started with equipment preparation until the last collection of milk.

Equipment needed in this study such as jug for milk collection, the syringe for measuring the milk and kefir grain, sieve tube and the bottles were provided by UNISZA laboratory and farm.

3.3 Experimental design

3.3.1 Milk collection and storage

The Saanen and Toggurnburg female goats were identified and analysed for milk collection purpose and will be separated with others goat in one small barn. Then, milk was collected from goats using manual milking process without using milking equipment. Approximately the milk collection was two litres in each collection. After that, the bottles were separated in two sample which are Kefir Milk (KM) and Fresh Milk (FM). All the bottles were filled up to 250ml and for (KM) samples only will added and mixed with 5ml of kefir grains. After that, all the samples were carried out and transported immediately to Veterinary Faculty of University Malaysia Kelantan at Pengkalan Chepa, Kelantan for physiochemical milk scanning. A Milko Scan, the machine was used to determined nutritional value between two samples; (KM) and (FM). After finished, the (KM) samples will incubated in room temperature in laboratory racks for the reaction of microorganism between kefir grain and fresh milk and for (FM) will incubated in freezer with 4^0C. After 72 hours, all the sample will be repeated again for scanning purpose to check up nutritional value (fat, protein and lactose) in each sample. These sample will have created as second collection. The triplicate sample collection will scan at Day 1, Day 4 and Day 8 of the storage time.

3.3.2 Milko Scan Mars method

A Milko Scan TM Mars puts powerful mid-infrared (FTIR) analytical technology within the scope of small to medium-sized dairies, helping them avoid sluggish and labor-intensive conventional testing methods and enhancing their ability to detect intentional or accidental milk supply adulteration. This was a simple way to analysed the milk and get instant the quality control. Firstly, simply place the sample under the pipette and press Start to get analytical results of up to six parameters in just one minute. Keep costs down with a robust flow system and no chemicals or disposables needed. The six parameters were fat, protein, lactose, total solids, solids non-fat, freezing point (milk only), but in this study just used three of parameters that were lactose, fat and protein which were necessary in milk component and for consumer. In addition, this machine able to check milk for known adulterants and anything that looks abnormal at the same time as performing other quality checks.

3.3.3 Data analysis

Data analysis on nutritional value content was done in triplicate samples. The data were collected and analysed by using T-test in Microsoft Excel. T-test is used to determine if there was a significant difference between the means of two groups. All data were expressed as mean ± standard error of mean (SEM) in bar graph. The single factor ANOVA was used to determine the p-value between 3 parameters of sample.

CHAPTER 4

RESULT AND DISCUSSION

4.1 The percentage of lactose between fresh milk (FM) and kefir milk (KM) in Day 1, 4 and 8

Figure 4.1 shows the percentage of lactose between fresh milk (FM) and kefir milk (KM) in Day 1, 4 and 8 that the (FM) is higher than (KM) (4.31 ± 0.08 vs 4.22 ± 0.08) in day 1 of the storage. In day 4, the lactose value in (KM) was decreasing from day 1 (4.22 ± 0.08) to (3.53 ± 0.14). the lactose in (FM) also was slightly decreased from day 1 to day 4 (4.31 ± 0.08) (4.28 ± 0.1). In day 8 the lactose value in (KM) was significantly decreased from day 4 to day 8 (3.53 ± 0.14 vs 2.96 ± 0.17) but in (FM) was increasing from day 4 to day 8 (4.28 ± 0.1 vs 4.36 ± 0.08). This data was shown that the lactose value in (KM) from day 1 to day 8 were significantly decreased and this result was necessary for person who have lactose intolerance symptoms which cannot consume directly the milk.

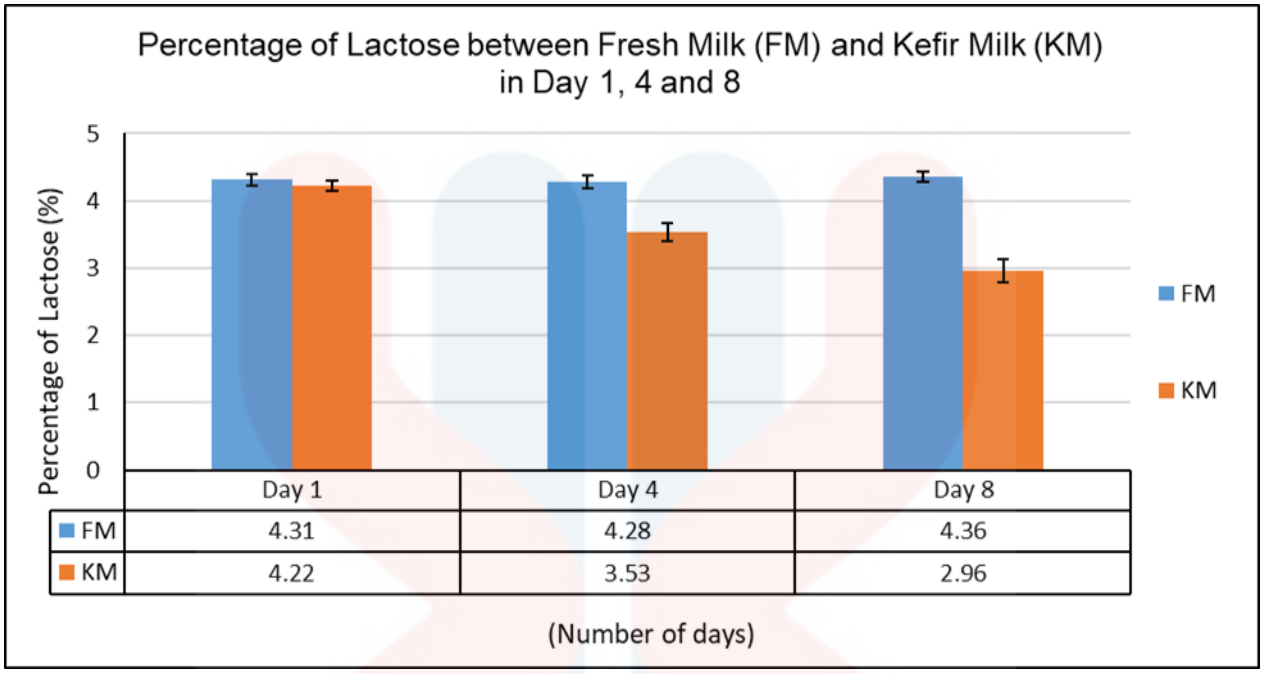


Figure 4.1: The percentage of lactose between (FM) and (KM) in day 1, 4 and 8

Table 4.1: P-value, mean and standard error of mean (SEM) of lactose percentage between (FM) and (KM) in Day 1, 4 and 8

Day	Lactose % in FM (Mean ± SEM)	Lactose % in KM (Mean ± SEM)	P-value
1	4.31 ± 0.08	4.22 ± 0.08	0.42
4	4.28 ± 0.1	3.53 ± 0.14	0.0001
8	4.36 ± 0.08	2.96 ± 0.17	0.0001

4.2 The percentage of fat between fresh milk (FM) and kefir milk (KM) in Day 1, 4, 8

Figure 4.2 shows the percentage of fat between fresh milk (FM) and kefir milk (KM) in Day 1, 4 and 8. The results were indicated fat content in (FM) was higher than fat content of (KM) in day 1 (4.31 ± 0.32 vs 4.2 ± 0.07). On day 4, the fat content in (FM) was increasing from day 1 to day 4 (4.31 ± 0.32 vs 4.81 ± 0.39) while in (KM) also was increasing from day

1 to day 4 (4.2 ± 0.07 vs 4.73 ± 0.10) but the fat content in (FM) started to decrease from day 4 to day 8 (4.81 ± 0.39 vs 3.97 ± 0.24).

Therefore, based on the result recorded, day 4 was the peak of fat level in (FM) meanwhile the fat content in (KM) from day 1 to day 8 were significantly increase (4.2 ± 0.07 vs 4.73 ± 0.10 vs 5.15 ± 0.09). It was shown that the (KM) was very suitable in butter or cheese making regarding it fat level increasing in storage time.

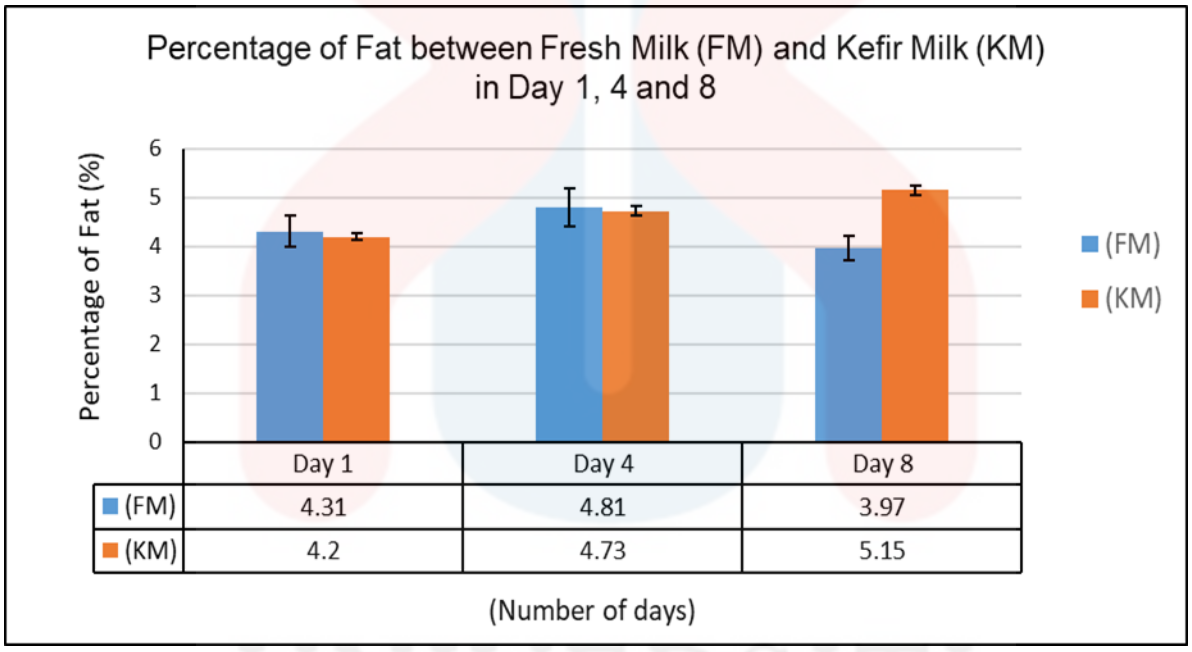


Figure 4.2: The percentage of lactose between (FM) and (KM) in day 1, 4 and 8

Table 4.2: P-value, mean and standard error of mean (SEM) of fat percentage between (FM) and (KM) in Day 1, 4 and 8

Day	Fat % in FM (Mean ± SEM)	Fat % in KM (Mean ± SEM)	P-value
1	4.31 ± 0.32	4.2 ± 0.07	0.76
4	4.81 ± 0.39	4.73 ± 0.10	0.27
8	3.97 ± 0.24	5.15 ± 0.09	0.003

4.3 The percentage of protein between fresh milk (FM) and kefir milk (KM) in day 1, 4 and 8

Figure 4.3 shows the percentage of protein between fresh milk (FM) and kefir milk (KM) in day 1, 4 and 8. The bar graph was indicated the higher of protein level in (KM) than (FM) on day 1 that was (3.53 ± 0.20 vs 3.23 ± 0.22). On day 4, it was similar with day 1 which protein content in (KM) was higher than (FM) in (3.76 ± 0.12 vs 3.29 ± 0.36) value. Moreover, the percentage of protein in (KM) was significantly increased from day 1 to day 8 (3.53 ± 0.20 vs 3.76 ± 0.12 vs 4.18 ± 0.23) compare to (FM), (3.23 ± 0.22 vs 3.29 ± 0.36 vs 3.27 ± 0.28). Based on this result, it revealed that (KM) was important in strong bone structure growth and osteoporosis disease.

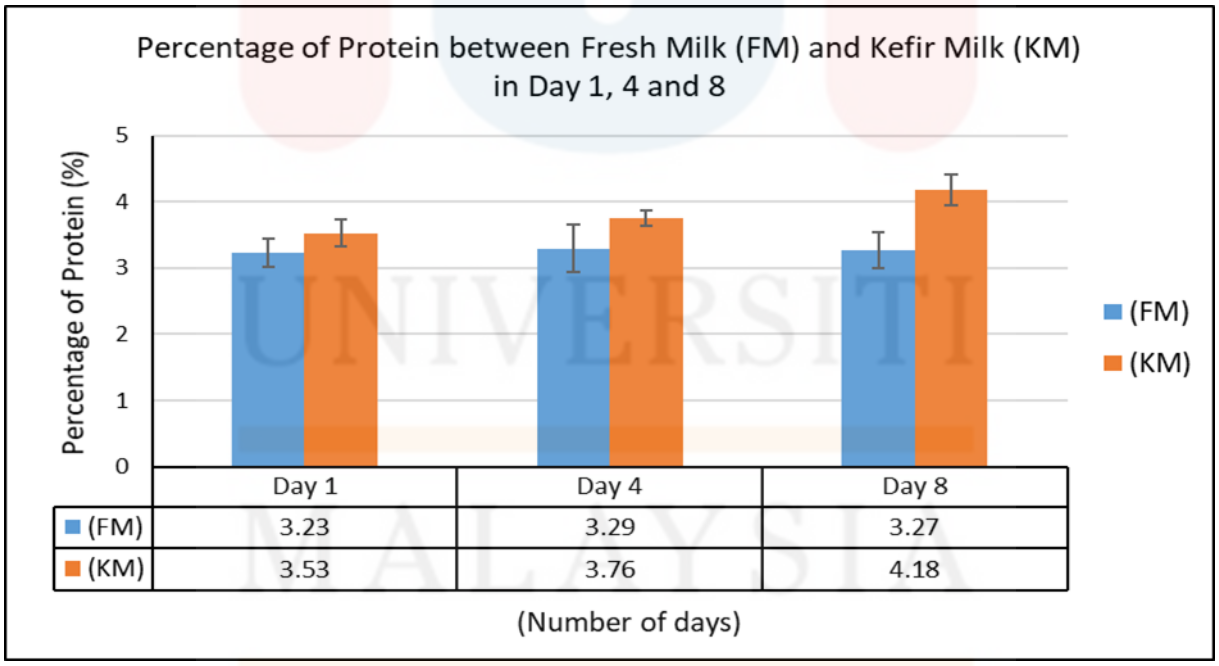


Figure 4.3: The percentage of protein between (FM) and (KM) in day 1, 4 and 8

Table 4.3: P-value, mean and standard error of mean (SEM) of protein percentage between (FM) and (KM) in Day 1, 4 and 8

Day	Protein % in FM (Mean ± SEM)	Protein % in KM (Mean ± SEM)	P-value
1	3.23 ± 0.22	3.53 ± 0.20	0.53
4	3.29 ± 0.36	3.76 ± 0.12	0.04
8	3.27 ± 0.28	4.18 ± 0.23	0.0008

4.4 The percentage of fat, protein and lactose on day 1, 4 and 8 in fresh milk (FM)

Figure 4.4 shows the percentage of fat, protein and lactose on day 1, 4 and 8 in (FM) sample. The results were comparing the fat, protein and lactose in different day of storage; day 1, 4 and 8. The fat content was increasing from day 1 to day 4 (4.31 ± 0.32 vs 4.81 ± 0.39) but statistically decreased from day 4 to day 8 (4.81 ± 0.39 vs 3.97 ± 0.24). Then, the protein level was slightly inclined from day 1 to day 4 (3.23 ± 0.07 vs 3.29 ± 0.10) but slightly declined from day 4 to day 8 (3.29 ± 0.10 vs 3.27 ± 0.09). the lactose value also was slightly decreased from day 1 to day 4 (4.31 ± 0.08 vs 4.28 ± 0.1) and slightly increased from day 4 to day 8 (4.28 ± 0.1 vs 4.36 ± 0.08). The study has indicated that fat content will decrease by the storage time but protein and lactose level will increase by storage time.

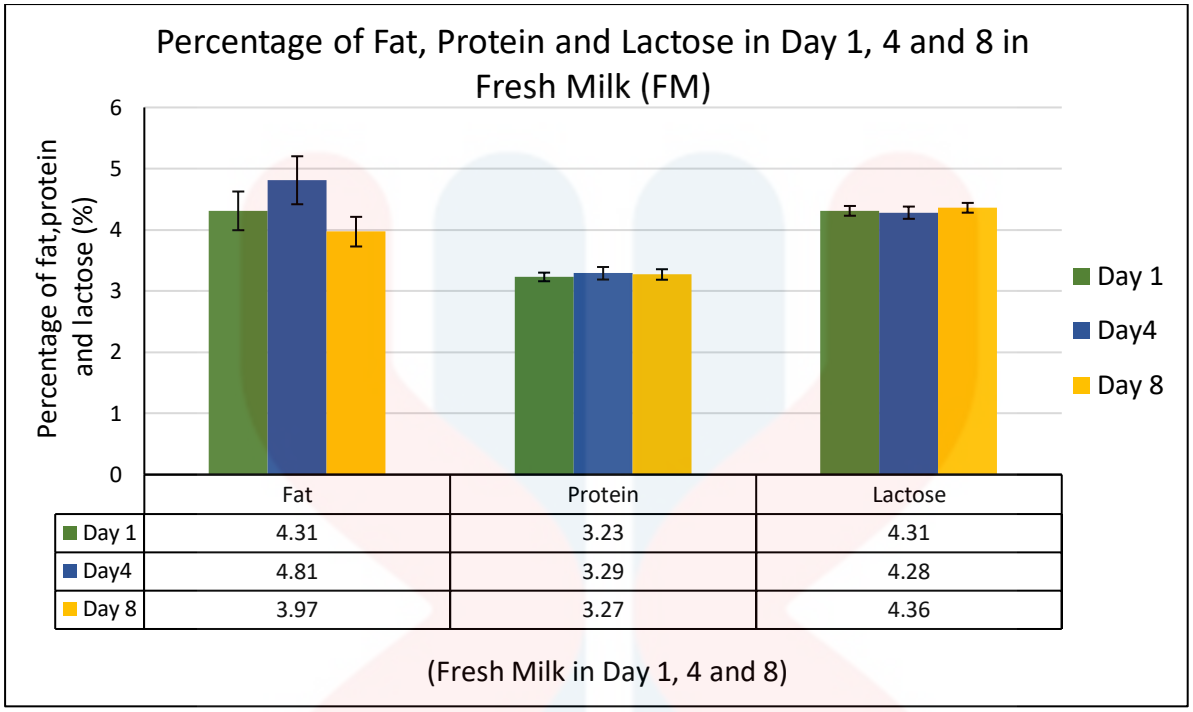


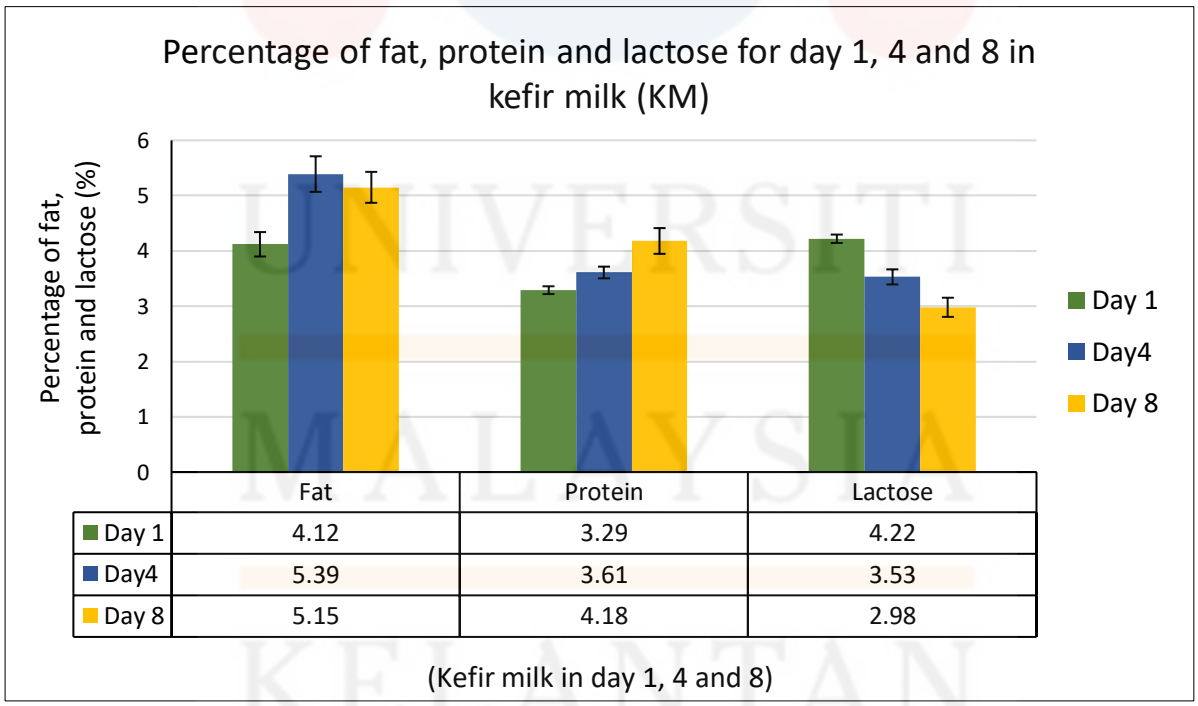
Figure 4.4: The percentage of fat, protein and lactose in day 1, 4 and 8 in (FM)

Table 4.4: P-value, mean and standard error of mean (SEM) of percentage of fat, protein and lactose in Day 1, 4 and 8 in (FM)

Day	Fat % in FM (Mean ± SEM)	Protein % in FM (Mean ± SEM)	Lactose % in FM (Mean ± SEM)	P-value
1	4.31 ± 0.32	3.23 ± 0.07	4.31 ± 0.08	0.000146
4	4.81 ± 0.39	3.29 ± 0.10	4.28 ± 0.1	0.000174
8	3.97 ± 0.24	3.27 ± 0.09	4.36 ± 0.08	0.000029

4.5 The percentage of fat, protein and lactose on day 1, 4 and 8 in kefir milk (KM)

Figure 4.5 shows that the percentage of fat, protein and lactose on day 1, 4 and 8 in (KM). The figure was shown that fat content was increased from day 1 to day 4 (4.12 ± 0.22 vs 5.39 ± 0.32) but slightly decreased from day 4 to day 8 (5.39 ± 0.32 vs 5.15 ± 0.28). the protein level was significantly increasing from day 1 to day 8 (3.29 ± 0.07 vs 3.61 ± 0.11 vs 4.18 ± 0.23). Moreover, the lactose value started to decrease from day 1 to day 8 (4.22 ± 0.08 vs 3.53 ± 0.14 vs 2.98 ± 0.17) in order to degradation of lactose to glucose and galactose by fermentation process. The study was indicated fat content will decrease by storage time after day 8 similar with lactose content was significantly decrease by storage time caused by fermentation process between kefir grains and milk sample but protein content was significantly increase by storage time.



	Fat	Protein	Lactose
Day 1	4.12	3.29	4.22
Day4	5.39	3.61	3.53
Day 8	5.15	4.18	2.98

Figure 4.5: The percentage of fat, protein and lactose in day 1, 4 and 8 in (KM)

Table 4.5: P-value, mean and standard error of mean (SEM) of percentage of fat, protein and lactose in Day 1, 4 and 8 in (KM)

Day	Fat % in FM (Mean ± SEM)	Protein % in FM (Mean ± SEM)	Lactose % in FM (Mean ± SEM)	P-value
1	4.12 ± 0.22	3.29 ± 0.07	4.22 ± 0.08	0.0000137
4	5.39 ± 0.32	3.61 ± 0.11	3.53 ± 0.14	0.0000000297
8	5.15 ± 0.28	4.18 ± 0.23	2.98 ± 0.17	0.000000138

DISCUSSION

4.6 Lactose decreased significantly from Day 1 to Day 8

The first novel finding in this study was the value of lactose in kefir milk (KM) collected from goat female was significantly decreased from Day 1 to Day 8 (4.22 ± 0.08 vs 3.53 ± 0.14 vs 2.96 ± 0.17, respectively). This is due to the breakdown of lactose to glucose and galactose during fermentation process. Rosa *et al.*, (2017) reported that during the fermentation process, lactose from milk was converted to acid, resulting in a decrease of pH and increase in consistency.

Other than that, several studies have been conducted in humans investigating the consumption of kefir for gastrointestinal function and dysfunction. Approximately 30% of milk lactose was hydrolyzed by the galactosidase enzyme, that is responsible for the formation of glucose and galactose from lactose. The kefir consumption was very good for person who was suspected in lactose intolerance or lactose maldigestion since it contained lactic acid bacteria (LAB), which hydrolyses the concentration of lactose, thus reducing concentration of lactose in the drink (Dimidi *et al.*, 2019). The classical term of lactose intolerance (LI) has been applied to the production of gastrointestinal symptoms (gas, bloat, abdominal cramps and discomfort often associated with mushy to watery diarrhea and sometimes with nausea and vomiting) after large amounts of lactose-containing food have been consumed (Szilagyi and Ishayek, 2018). Moreover, Machado *et al.*, (2013) reported that the ability to reduce lactose levels and the presence of ß-galactosidase activity in fermented milk products make kefir very ideal consumed by people identified as intolerant to lactose. In

addition, Purnomo and Muslimin, (2012) also reported that the lactose content was decreased from 4.92% to 4.02% during the first 24 hours of fermentation and the pH also decreased to 4.2 %. They also stated that lactose content of goat milk kefir decreases with a higher amount of kefir grain added during preparation as well as a longer incubation period.

4.7 The significant value of fat and protein from Day 1 to Day 8

The second data shown the value of fat and protein in kefir milk increased from Day 1 to Day 8 (4.20 ± 0.22 vs 5.15 ± 0.28) and (3.53 ± 0.21 vs 4.18 ± 0.23). The protein and fat content of milk varies depending on the species, breed, lactation time, diet, health status of the udder and the environmental level. An increase the ethanol synthesis during lactose-alcohol fermentation in goat milk also significantly elevated a polyunsaturated fatty acid (PUFA), (D. Cais-Sokolińska et al., 2015). The study elaborated on synthesis of ethanol with elevated fatty acids. This indicated that raising of fat correlated with acidity value. Ertekin and Guzel-Seydim, (2010) also reported that the acidity values of kefir samples increased during storage while the pH value was decreased. The longer the storage time also gives the sample more acidic taste because it allowed the continuous process of fermented. This is regarding to the increase of probiotic microorganism's breakdown with increase in storage time.

Moreover, Vieira *et al.*, (2015) revealed that lactic acid bacteria can increase free fatty acid production through lipolysis of milk fat. They also stated that although pH decreased during fermentation, acidity and protein content increased, but not due to storage time. In contrast, Tomar *et al.*, (2019) was found that protein content decreased during storage of kefir samples produced from kefir grains.

In addition, they also reported that Kefir's dry matter content from cow, cattle, or goat milk changed by milk type. Besides, goat milk consists of more essential fatty acids (EFAs) and conjugated linoleic acids (CLAs) which are dimensional and structural isomers of linoleic acid, relative to other milks (Turkmen, 2017). The author also concluded that a more amount of CLAs in goat milk also good for the cases of malabsorption syndromes. Similar to cow's milk, goat's milk had a higher level of short chain fatty acid, smaller fat globules which made it easy to digest (Wang *et al.*, 2017b). In addition, Dorota Cais-Sokolińska *et al.*, (2015) reported the positive effect of false flax (Camelina sativa) cake in feeding goats on the fatty acid profile resulting in an increase in the proportion of PUFA, including CLA, n-3 fatty acids and monoenoic trans acids.

4.8 The effect of storage time

The storage time between Day 1 and Day 8 in fat, protein and lactose were significantly different whereby the lactose value was decreased while in fat and protein values were significantly increased. This was due to the degradation between kefir microorganism in sample. Kesenkaş et *al.*, (2017) reported that continuous metabolic activities of kefir microbiota during storage conditions may affect the nutritional composition of kefir sample. However, it was shown that fat content is not significant between kefir milk and fresh milk in Day 4. This can conclude that breakdown of fat from milk with lactic acid bacteria (LAB) requires more than 4 Days to achieve the significant value. Therefore, this study suggested that Day 8 (192 h) is the best storage time for fermentation in kefir.

O'Brien *et al.*, (2016) reported that during the processing of kefir with grains, the fermentation reaction of lactic acid slows or stops extremely as the pH decreases, but the

fermentation of yeast continues, enabling the production of ethanol to increase during storage. The result will varies depending on different temperature, microbial population in kefir grains and storage time. Other than that, derivation of milk from different species (buffalo) will affect the storage time due to higher microbial enumeration (Atalar *et al.*, 2015). In addition, the higher the microbial population on sample, the shorter the time taken of reaction between LAB, yeast and kefir grains because the grain of kefir will consume these good types of bacteria and yeast for the whole fermentation process. The storage time also depends on the weight of kefir grains (gram) mixed with milk sample.

Moreover, Purnomo and Muslimin, (2012) reported the reduction of sugar during the fermentation of kefir may also have been due to its incubation time. However, regardless of the time of storage, there were no differences in fat, protein, lactose and mineral content between kefirs, no increase in active acidity was found during refrigerated storage of kefir (Cais-Sokolińska *et al.*, 2015).

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

In conclusion, the lactose decreased significantly from Day 1 to Day 8 but differs in the fat and protein values that were significantly increased. However, the results were not significant when comparing between breeds. Moreover, the effect of storage time also important in kefir sample. Many factors can determine this result such as feeding management and lactation period. Moreover, the storage time was an important parameter in this study because it affected the results. The fermentation between kefir grains and any type of bacteria and yeast (especially LAB) in milk had different growing time depending on the concentration of microorganism in milk sample and kefir grains. The higher the concentration of microorganisms in milk sample and kefir grain, the faster the growing time for fermentation to occur. This study concluded that the best time to get a good result in the reaction of kefir grains with milk sample is on the eighth day.

5.2 RECOMMENDATION

This study has many potentials for future works in regard to kefir production, kefir manufacture, kefir storage and kefir microbial profile. This study looked into the values of nutritional values on Day 1, Day 4 and Day 8. Results at Day 4 were insignificant, but data obtained at Day 8 were promising. Thus, investigation between Day 4 to Day 8 was suggested to look for the best time storage on kefir fermentation.

Another recommendation was the bacteria plate count. Normally, in kefir fermentation most have good probiotic bacteria such as lactic acid bacteria (LAB) and yeast. But, for the good kefir fermentation product must having a higher concentration of probiotic bacteria to ensure the results have a numerous of benefits on health.

Moreover, the pH value also effected in kefir production. Many researchers reported with decrease in pH value will interfere the reaction of microorganisms in sample. So in the processing of kefir, the pH value must be considering as vital component in kefir product.

In addition, organoleptic test; milk evaluation to test the colour, textures and smell of kefir samples. This test is important to improve kefir product. Most company regarding on food will use this test to refine their product. With sensory testing, a producer can check the effectiveness of the product mostly on kefir because kefir normally in slightly tartness taste whereby not many people are like to consume it.

The last but not least, this study was not following the procedure in milk processing such as standardization, pasteurization and homogenization. The study just did milk fermentation processing between milk sample and kefir grains. Thus, the suggestion was included all the milk processing procedure to maintain the product shelf-life and for production purpose.

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APPENDIX A

Figure A1: Milk sampling at Pasir Akar Farm, Besut

Figure A2: Cleaning the goat's nipples before milk sampling

Figure A.3: Packaging the milk samples into bottles following fresh milk (FM) and kefir milk (KM) samples

Figure A.4: Saanen goat female for milk sampling

Figure A5: Milk samples placed in icebox were transported for Milko-Scan scanning at Veterinarian Faculty, UMK

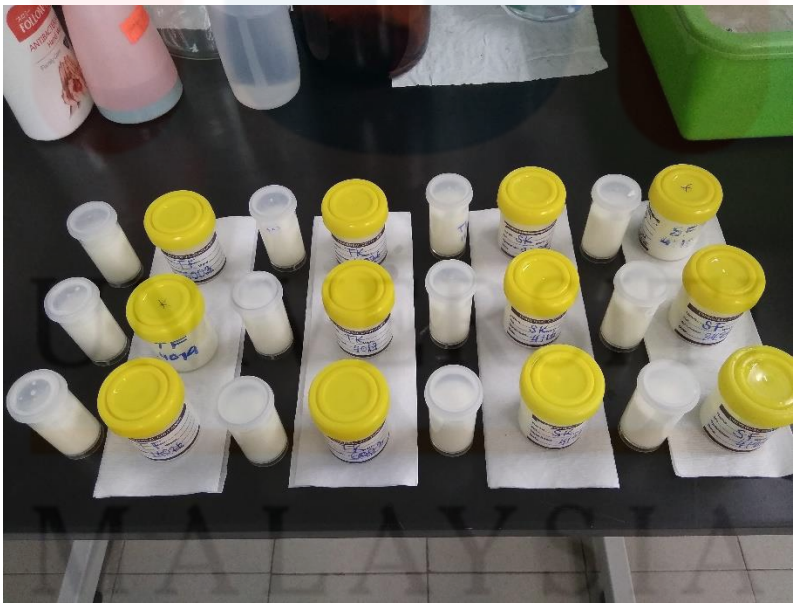


Figure A6: Milk samples were placed in small bottles before scanning

Figure A7: Milk samples were tested using Milko-scan

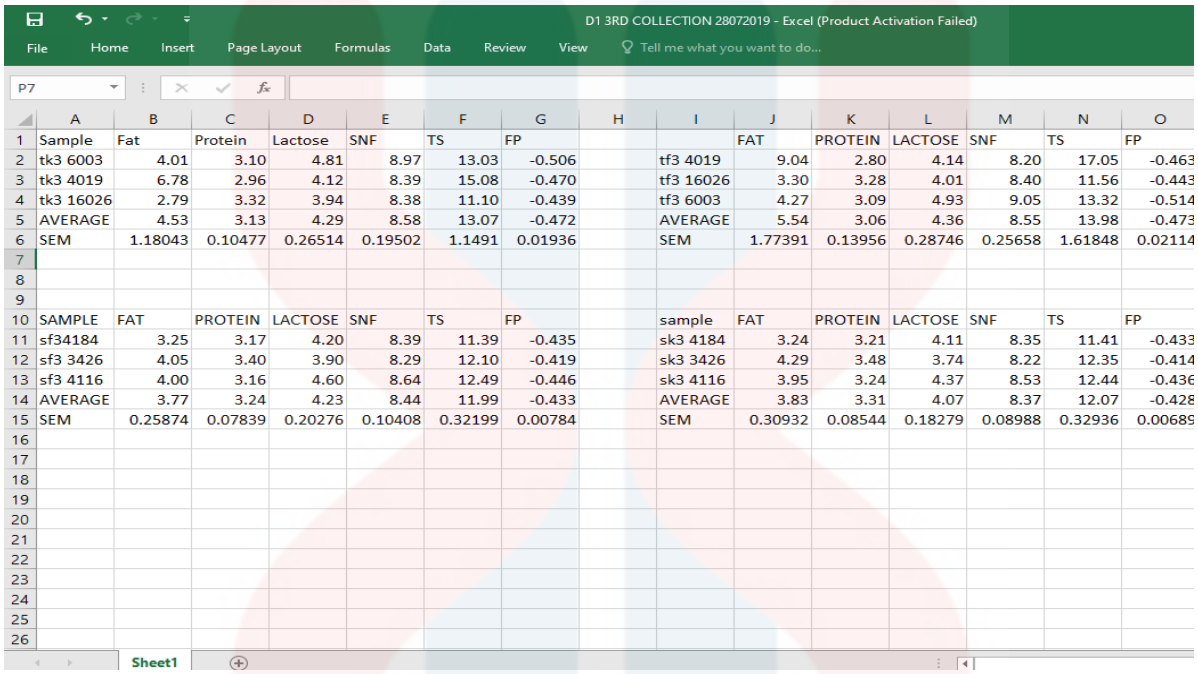
Figure A8: Milk samples were incubated in UniSZA laboratory for 8 days

Figure A9: The example of Kefir products in Malaysia markets, 'MayKef'

Figure A10: A Bronze medal was awarded in Youthpreneur Revolution and Innovation Challenge 2019 on 'Nutritional Value of Kefir derived from goat's milk'

APPENDIX B

Table B1: The sample collection for day 1 in Excel sheets



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	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	Sample	Fat	Protein	Lactose	SNF	TS	FP			FAT	PROTEIN	LACTOSE	SNF	TS	FP
2	tk3 6003	4.01	3.10	4.81	8.97	13.03	-0.506		tf3 4019	9.04	2.80	4.14	8.20	17.05	-0.463
3	tk3 4019	6.78	2.96	4.12	8.39	15.08	-0.470		tf3 16026	3.30	3.28	4.01	8.40	11.56	-0.443
4	tk3 16026	2.79	3.32	3.94	8.38	11.10	-0.439		tf3 6003	4.27	3.09	4.93	9.05	13.32	-0.514
5	AVERAGE	4.53	3.13	4.29	8.58	13.07	-0.472		AVERAGE	5.54	3.06	4.36	8.55	13.98	-0.473
6	SEM	1.18043	0.10477	0.26514	0.19502	1.1491	0.01936		SEM	1.77391	0.13956	0.28746	0.25658	1.61848	0.02114
7															
8															
9															
10	SAMPLE	FAT	PROTEIN	LACTOSE	SNF	TS	FP		sample	FAT	PROTEIN	LACTOSE	SNF	TS	FP
11	sf34184	3.25	3.17	4.20	8.39	11.39	-0.435		sk3 4184	3.24	3.21	4.11	8.35	11.41	-0.433
12	sf3 3426	4.05	3.40	3.90	8.29	12.10	-0.419		sk3 3426	4.29	3.48	3.74	8.22	12.35	-0.414
13	sf3 4116	4.00	3.16	4.60	8.64	12.49	-0.446		sk3 4116	3.95	3.24	4.37	8.53	12.44	-0.436
14	AVERAGE	3.77	3.24	4.23	8.44	11.99	-0.433		AVERAGE	3.83	3.31	4.07	8.37	12.07	-0.428
15	SEM	0.25874	0.07839	0.20276	0.10408	0.32199	0.00784		SEM	0.30932	0.08544	0.18279	0.08988	0.32936	0.00689
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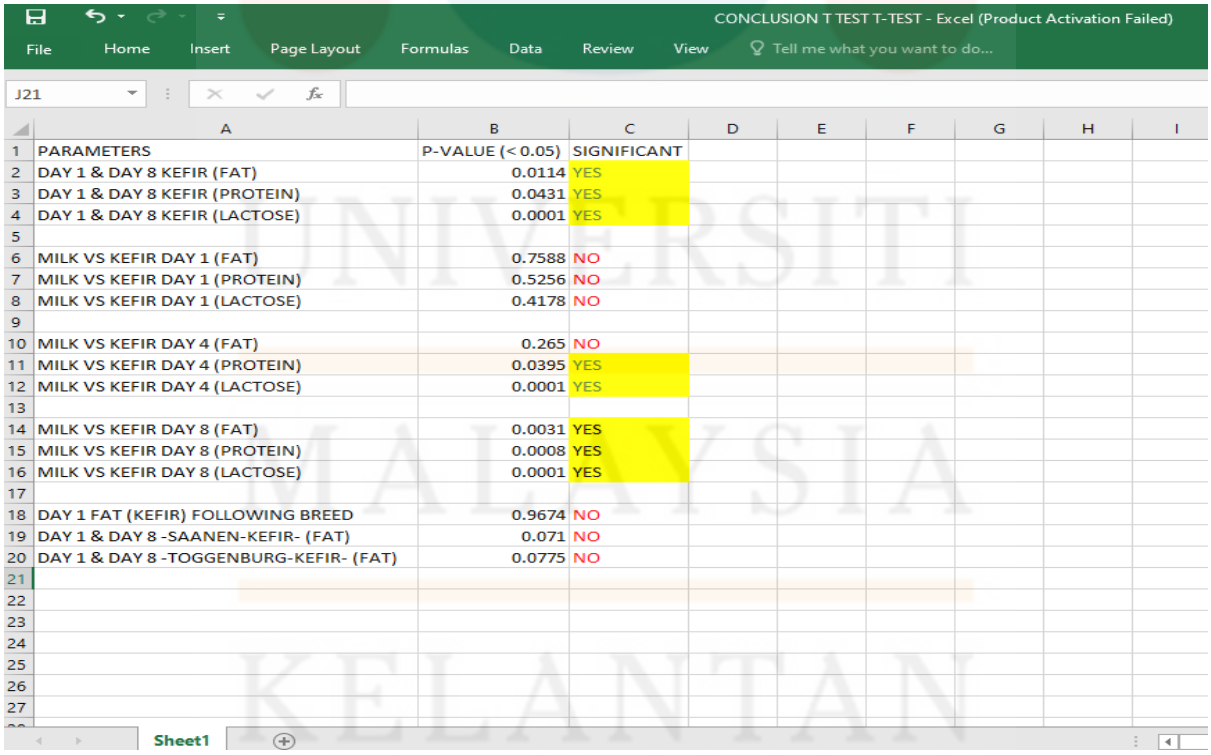
Sheet1

Table B2: T-test results for all collection in Excel sheets



CONCLUSION T TEST T-TEST - Excel (Product Activation Failed)

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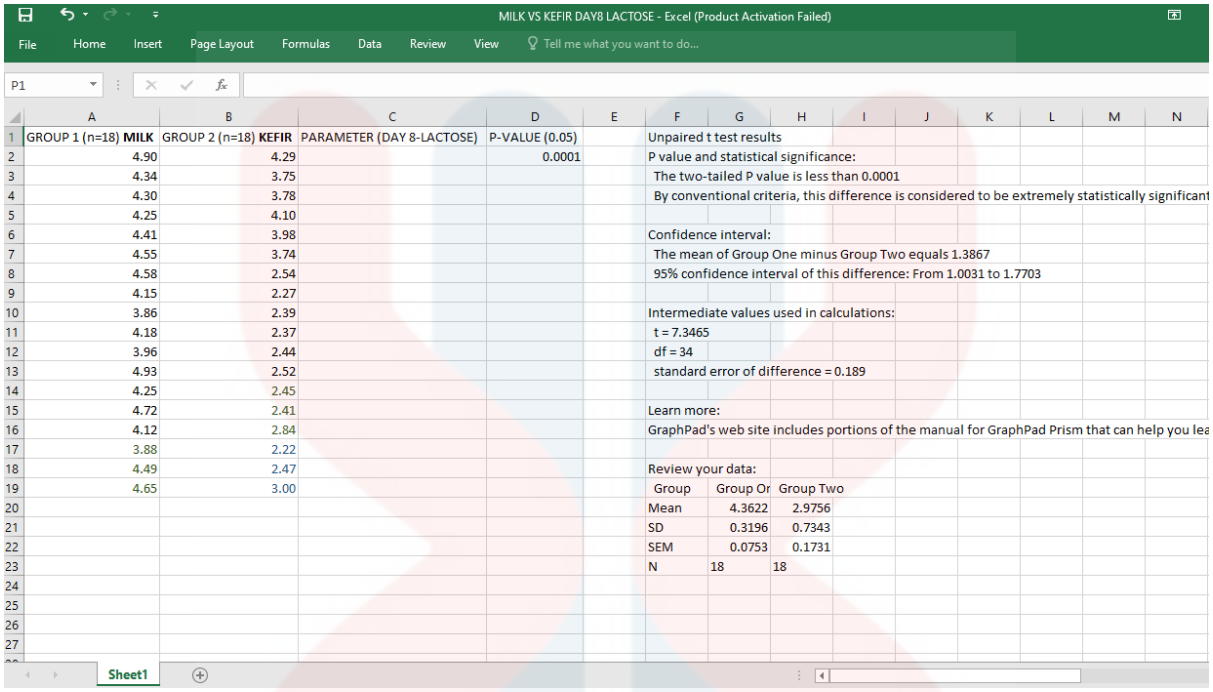
	A	B	C	D	E	F	G	H	I
1	PARAMETERS	P-VALUE (< 0.05)	SIGNIFICANT						
2	DAY 1 & DAY 8 KEFIR (FAT)	0.0114	YES						
3	DAY 1 & DAY 8 KEFIR (PROTEIN)	0.0431	YES						
4	DAY 1 & DAY 8 KEFIR (LACTOSE)	0.0001	YES						
5									
6	MILK VS KEFIR DAY 1 (FAT)	0.7588	NO						
7	MILK VS KEFIR DAY 1 (PROTEIN)	0.5256	NO						
8	MILK VS KEFIR DAY 1 (LACTOSE)	0.4178	NO						
9									
10	MILK VS KEFIR DAY 4 (FAT)	0.265	NO						
11	MILK VS KEFIR DAY 4 (PROTEIN)	0.0395	YES						
12	MILK VS KEFIR DAY 4 (LACTOSE)	0.0001	YES						
13									
14	MILK VS KEFIR DAY 8 (FAT)	0.0031	YES						
15	MILK VS KEFIR DAY 8 (PROTEIN)	0.0008	YES						
16	MILK VS KEFIR DAY 8 (LACTOSE)	0.0001	YES						
17									
18	DAY 1 FAT (KEFIR) FOLLOWING BREED	0.9674	NO						
19	DAY 1 & DAY 8 -SAANEN-KEFIR- (FAT)	0.071	NO						
20	DAY 1 & DAY 8 -TOGGENBURG-KEFIR- (FAT)	0.0775	NO						
21									
22									
23									
24									
25									
26									
27									

Sheet1

Table B3: Unpaired t-test results with (FM) vs (KM) in day 8 (lactose) in Excel sheets



MILK VS KEFIR DAY8 LACTOSE - Excel (Product Activation Failed)

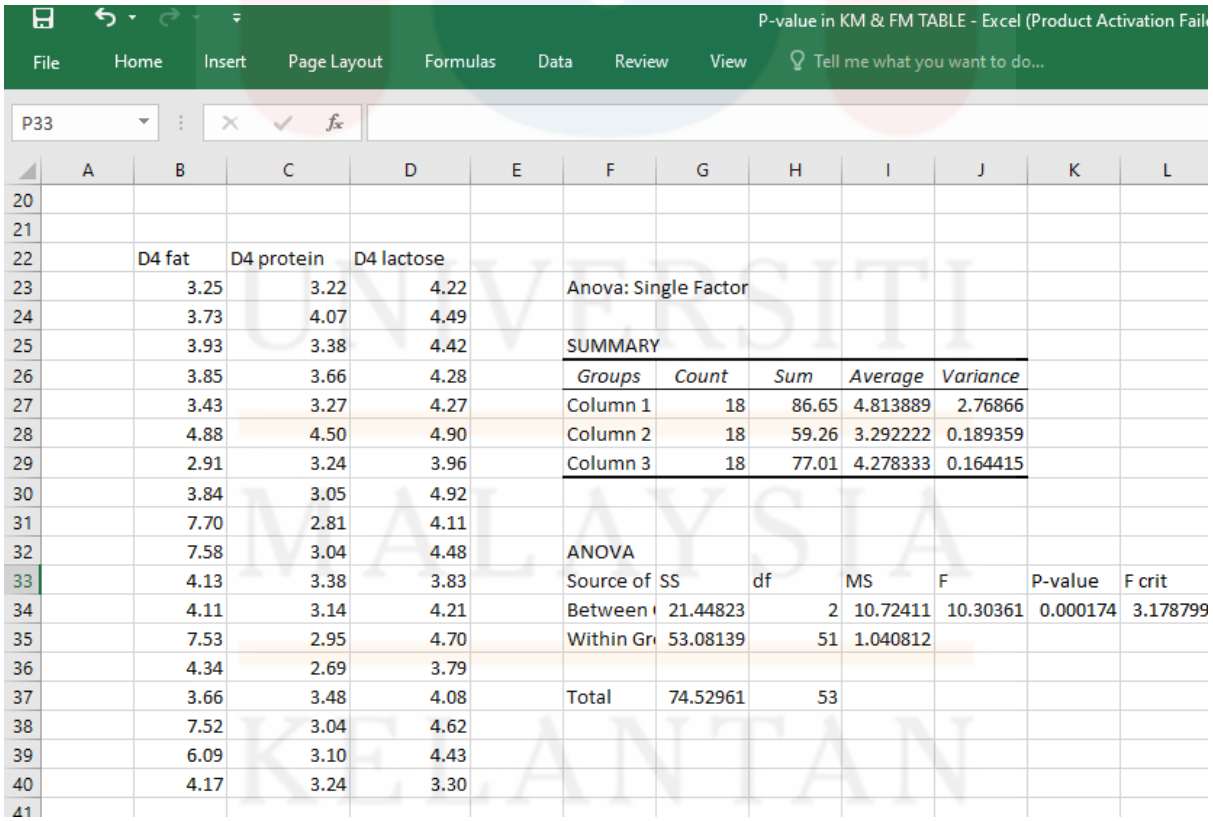
File Home Insert Page Layout Formulas Data Review View Tell me what you want to do...

P1

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	GROUP 1 (n=18) **MILK**	GROUP 2 (n=18) **KEFIR**	PARAMETER (DAY 8-LACTOSE)	P-VALUE (0.05)		Unpaired t test results								
2	4.90	4.29		0.0001		P value and statistical significance:								
3	4.34	3.75				The two-tailed P value is less than 0.0001								
4	4.30	3.78				By conventional criteria, this difference is considered to be extremely statistically significant								
5	4.25	4.10												
6	4.41	3.98				Confidence interval:								
7	4.55	3.74				The mean of Group One minus Group Two equals 1.3867								
8	4.58	2.54				95% confidence interval of this difference: From 1.0031 to 1.7703								
9	4.15	2.27												
10	3.86	2.39				Intermediate values used in calculations:								
11	4.18	2.37				t = 7.3465								
12	3.96	2.44				df = 34								
13	4.93	2.52				standard error of difference = 0.189								
14	4.25	2.45												
15	4.72	2.41				Learn more:								
16	4.12	2.84				GraphPad's web site includes portions of the manual for GraphPad Prism that can help you lea								
17	3.88	2.22												
18	4.49	2.47				Review your data:								
19	4.65	3.00				Group	Group Or	Group Two						
20						Mean	4.3622	2.9756						
21						SD	0.3196	0.7343						
22						SEM	0.0753	0.1731						
23						N	18	18						
24														
25														
26														
27														

Sheet1

Table B4: Single-factor ANOVA was used to determine p-value between storage days in Excel sheets



P-value in KM & FM TABLE - Excel (Product Activation Fail

File Home Insert Page Layout Formulas Data Review View Tell me what you want to do...

P33

	A	B	C	D	E	F	G	H	I	J	K	L
20												
21												
22		D4 fat	D4 protein	D4 lactose								
23		3.25	3.22	4.22		Anova: Single Factor						
24		3.73	4.07	4.49								
25		3.93	3.38	4.42		SUMMARY						
26		3.85	3.66	4.28		*Groups*	*Count*	*Sum*	*Average*	*Variance*		
27		3.43	3.27	4.27		Column 1	18	86.65	4.813889	2.76866		
28		4.88	4.50	4.90		Column 2	18	59.26	3.292222	0.189359		
29		2.91	3.24	3.96		Column 3	18	77.01	4.278333	0.164415		
30		3.84	3.05	4.92								
31		7.70	2.81	4.11								
32		7.58	3.04	4.48		ANOVA						
33		4.13	3.38	3.83		Source of	SS	df	MS	F	P-value	F crit
34		4.11	3.14	4.21		Between (	21.44823	2	10.72411	10.30361	0.000174	3.178799
35		7.53	2.95	4.70		Within Gr	53.08139	51	1.040812			
36		4.34	2.69	3.79								
37		3.66	3.48	4.08		Total	74.52961	53				
38		7.52	3.04	4.62								
39		6.09	3.10	4.43								
40		4.17	3.24	3.30								
41												