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TED ANKARA COLLEGE FOUNDATION HIGH SCHOOL

EXTENDED ESSAY

Differences in the total number of colonies that resist synthetic stomach environment of homemade and different brand of kefir samples

Research Question: Will there be a significant difference between the number of alive bacteria in homemade kefir and the 4 different brands of kefir bought from the store, with regard to their probiotic effects after incubating the cell suspensions from each sample at pH 2 by using Cell Viability Assay method?

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ABSTRACT

The aim of my extended essay is to investigate the probiotic benefits of homemade kefir and the four different brands (**Ülker İçim, Altınkılıç, AOÇ, Eker**) of kefir which can be easily found in the supermarkets exposed to synthetic stomach environment.

Figure1: Kefir Grains

The research question is: “Will there be a significant difference between the number of alive bacteria in homemade kefir and the four different brands of kefir bought from the store, with regard to their probiotic effects after incubating the cell suspensions from each sample at pH 2 by using Cell Viability Assay method?”

My hypothesis is: “There is a considerable difference between the number of alive bacteria in homemade kefir and the four different brands of kefir bought from the supermarket after being exposed to stomach acid, known as hydrochloric acid. Homemade kefir will have the highest number of alive bacteria that resist stomach acid, while the other kefir types which are sold in the supermarkets will have lesser number of alive bacteria.”

For the purpose of examining the hypothesis and answering the research question, Cell Viability Assay method was used. All homogenized 10 ml of five kefir samples were exposed to hydrochloric acid (pH: 2) and incubated for four hours at 37 °C. After incubation, serial dilutions were prepared with kefir samples and spread all over the petri dishes. The dishes were incubated for 72 hours at 30°C in an incubator. Lastly, the number of colonies were counted and results were compared to each other.

The final results of the experiment indicated that, **Ülker İçim** has the highest mean number of alive bacteria colonies (**313**) left after being exposed to hydrochloric acid. **The homemade kefir** which was expected to have the largest number of alive bacteria, is in the second rank (**296**). **Altınkılıç** (strawberry flavoured) is in the third place (**144**). **AOÇ** has taken the fourth place (**118**), followed by **Eker** which has the lowest number of alive bacteria (**49**).These results showed that the homemade kefir doesn't have the highest number of alive bacteria after being treated with hydrochloric acid and this doesn't support the hypothesis. Hence, the mean results demonstrate that there is slight difference between **Ülker İçim** and the **homemade kefir** with regard to the number of alive bacteria. According to the ANOVA test results, there is a significant mean difference between the number of alive bacteria colonies in homemade and four different brands of kefir. The p-value of the test is $6.21x10^{-18}$, which is smaller than 0.05.

Word Count: 414

Shavit, Elinoar. "Renewed Interest in Kefir, the Ancient Elixir of Longevity." *American Journal of Ophthalmology* 86.2 (2008)

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I. INTRODUCTION AND BACKGROUND INFORMATION

I observe that my parents always attach importance to healthy diet and consuming nutritious food. My mother tries to select the most beneficial food and prepare well balanced and healthy meals for us. My father frequently reads articles about healthy eating and informs us about those newly found research results related with some sort of healthy food in order to have well balanced diet at home. So my parents always keep kefir at home and we drink kefir almost every night.

Kefir is a well-known drink which is commonly called as “miracle drink” due to its various health benefits mainly on the immune and intestinal system of the human body. Kefir is the product of the fermentation of milk with kefir grains and mother cultures prepared from grains.[1] Kefir grains are irregularly shaped, gelatinous masses varying in size from 1 to 6 mm in diameter.[1] These grains contain lactic acid bacteria (lactobacilli, lactococci, leuconostocs), acetic acid bacteria and yeast mixture coupled together with casein and complex sugars by a matrix of polyssacharide.[1] With the help of these probiotic bacteria, kefir contributes to the treatment of digestive diseases and lactose intolerance problems. The probiotics which are frequently called as “superfoods” consisting of alive microorganisms contribute to the body systems with their healing and improving effects. One of the criteria for probiotic bacteria is that they should be able to withstand the harsh conditions of the gastrointestinal tract, including extreme pH conditions present in the stomach and the action of bile salts and digestive enzymes.[2] Kefir is one of the most beneficial food in terms of probiotics which helps the helpful bacteria get into body systems and helps improving the microbial harmony in the intestinal system. The microbiological and chemical composition of kefir indicates that it is a much more complex probiotic, as the large number of different bacteria and yeast found in it distinguishes it from other probiotic products.[3] Kefir, because it is milk based, is able to buffer the pH of the stomach when ingested and thereby provide time for many of the bacteria to pass through to the upper small intestine.[3]

Aside from these benefits, kefir is a very useful drink for preventing the harms of such diseases as, asthma, bronchitis, hypertension, diabetes, colitis etc. Kefir can reduce the risk for certain cancers including colon cancer, by preventing the growth of cancerous cells.[4] Kefir has antioxidants and anti-aging properties.[4] It can

neutralize the free radicals, that damage the body cells and tissues by oxidizing them.[4] By reducing the impact of free radical damage, kefir can slow down the aging process.[4]

When I was a little child, I didn't like the taste of kefir. As a little child, I couldn't understand the importance of drinking kefir. As I have grown up, I have read about the benefits of kefir and was very surprised to learn how miraculous kind of a drink kefir is. Sometimes my mother isn't able to prepare kefir from homemade grains, then we always buy a specific brand of kefir (**Eker**) as we like the taste of it and often buy other products of that brand like yoghurt and ayran. On the other hand, I started to think about whether the homemade kefir containing no additives is much more beneficial than the packaged kefir products that are sold in the supermarkets. I also thought that at the times we weren't able to prepare kefir at home; apart from **Eker** could we buy a different brand? Which brand was as effective as homemade kefir; which packaged kefir product was the most beneficial in terms of the number of alive bacteria? Which brand could resist stomach pH most?

Therefore, I decided to examine four different brands of kefir sold in supermarkets and compare them with each other and with the homemade kefir sample in terms of the number of alive bacteria that resist stomach acid.

I formed the research question as: "Will there be a significant difference between the number of alive bacteria in homemade kefir and the 4 different brands of kefir bought from the store, with regard to their probiotic effects after incubating the cell suspensions from each sample at pH 2 by using Cell Viability Assay method?"

II. HYPOTHESIS

Kefir is prepared by mixing milk with kefir grains. There are several major strains of bacteria found in different types of kefir.[5] These may include *Lactobacillus caucasus* and *Leuconostoc*, *Acetobacter*, and *Streptococcus* species.[5] Kefir also contains yeasts, such as *Saccharomyces*, *Klyuveromyces* and *Torulopsis*, many vitamins, minerals, enzymes and amino acids.[5] Some of the essential nutrients found in kefir include vitamins A, B2, B12, D and K; the minerals calcium, magnesium, and phosphorus; and the amino acid tryptophan.[5] Nowadays many people attach much importance to having a healthy life and consuming healthy food. Therefore kefir has become one of the most popular healthy food types. When I started to study for my extended essay and for my experiment, I examined the ingredients and nutritive values of kefir types sold in the supermarkets.

The nutritive values involved in these kefir brands may affect the consumers' preferences and this can be reflected through the sales numbers. Unequivocally, a varied type of consumers buy kefir and drink it regularly just because they are aware of the benefits of this "miracle drink" on the body health which is known to contain alive and probiotic bacteria which could resist to the influences of pH level in the stomach. On the other hand, Commercial Kefir found in stores is limited by the bottling process.[5] Companies need to suppress or halt yeast fermentation and culturing in order to prevent continued carbonation or the bottles could explode.[5] This process leaves you with commercial kefir which, while still good, typically has mild and/or suppressed culture, and less varieties of bacteria and yeast.[5] Because of these production based factors and the limitations of shelf life the homemade kefir which is prepared without any additives or artificial ingredients and which is not being exposed to any shelf life process could be the richest in the number of alive bacteria. Thus, my aim was to investigate whether the homemade kefir contains more alive bacteria than the packaged brands of kefir sold in the supermarkets after exposed to synthetic stomach environment.

So, my hypothesis was formed as "There is a considerable difference between the number of alive bacteria in homemade kefir and the four different brands of kefir bought from the supermarket after being exposed to stomach acid, known as hydrochloric acid. Homemade kefir will have the highest number of alive bacteria that

resist stomach acid, while the other kefir types which are sold in the supermarkets will have lesser number of alive bacteria." My null hypothesis could be stated as; "There is no difference between the number of alive bacteria in homemade kefir and the four different brands of kefir sold at the stores after being exposed to stomach acid."

III. METHOD DEVELOPMENT AND PLANNING

The hypothesis of my experiment is "There is a considerable difference between the number of alive bacteria in homemade kefir and the four different brands of kefir bought from the supermarket after being exposed to stomach acid, known as hydrochloric acid. Homemade kefir will have the highest number of alive bacteria that resist stomach acid, while the other kefir types which are sold in the supermarkets will have lesser number of alive bacteria." In order to test my hypothesis, I decided to use "Cell Viability Assay" method, which was the most suitable one to count the alive bacteria colonies after the exposure of HCl similar to gastric acid. Cell-based assays are often used for screening collections of compounds to determine if the test molecules have effects on cell proliferation or show direct cytotoxic effects that eventually lead to cell death.[6]

As stated in the definition of "Cell Viability Assay" method, the stability of the probiotic bacterium in kefir samples will be tested in the experiment, the highest number of bacteria colonies, between the four different kefir brands, will show the closest similarity with the homemade kefir. Thus, my independent variable is the type of kefir, homemade or store-bought, therefore my dependent variable is the number of alive bacteria present in each type of kefir after the exposure of gastric acid.

After deciding on my hypothesis and variables, I needed to choose the kefir brands to use in my experiment firstly. Since there are already 4 brands (**Ülker İçim, Altınkılıç, AOÇ and Eker**) known by the consumers and sold widely in the supermarkets, I bought one bottle from each brand by selecting the ones with the same expiry dates and from the same supermarket. Among these brands, **Altınkılıç** has a broad range of flavoured kefir types like strawberry, banana-honey, grape-apricot. Parents would prefer these flavoured kefir types in order to make their children like the taste of kefir. That is why I wanted to examine a kefir sample

containing additives like strawberry extract to demonstrate the usefulness of these types. On the other hand, I had no difficulty to find a sample of kefir made from homemade grains as there is always homemade kefir in our house. Thus, I gathered all the materials necessary for my experiment.

For the next stage, I needed to find a laboratory to conduct my experiment as this study requires a developed microbiology lab. So, I contacted with the laborants from Ankara Food Control Laboratory Directorate and got permission and help for the experiment. Before starting the main trials, I made a control trial to determine the number of bacterium present in each kefir sample without gastric acid. All kefir samples were shaked for the homogenization of the bacteria all over the test tube. One ml of each sample was inoculated to a medium, made up of 17.5 g Plate Count Agar which is equivalent to the medium recommended by the APHA (American Public Health Association) and the PHLS (Public Health Laboratory Service) for the plate count of micro-organisms in food, milk and other dairy products[7] and 1 g Skim Milk Powder which is used for cultivation and enumeration of microorganisms encountered in dairy industry.[8] This media supports the growth of bacteria in dairy products. To prevent the contamination caused from the outer environment, these mediums were autoclaved for 15 minutes at 121°C. Also, the disposable pipette tips and stripettes, which are attached to the pipette controller, were changed every time to prevent cross-contamination between the trials.

According to the ISO 4883-1 standards, the number of the mesophilic bacteria should be counted in plate count agar medium, with skim milk powder addition, and after the inoculation of the medium it should be incubated for 72 hours at 30°C. So all the incubations were done at 30°C for 72 hours.

The dilutions were prepared from 10^{-1} to 10^{-6} because in higher dilutions than 10^{-6}, the number of colonies reproduced is above the counting process. Accurate counting without any dilution, would make it impossible to count the colonies formed on agar plates. During the counting of incubated colonies, each colony is marked with water proof pencil to eliminate counting twice.

During the experiment, the mediums were inoculated to 12 petri dishes for one type of kefir, since five trials were conducted, 60 petri dishes were used. Thus, 300 petri dishes were used for all kefir types in total.

While performing the main trial, 9 ml of each sample was exposed to one ml of hydrochloric acid and incubated for four hours, which is the maximum time length a nutrient can stay in the stomach, at 37°C. HCl was chosen because it had the most similarities with the gastric acid. Since a synthetic stomach environment was meant to be created, HCl was the best choice. When all dilutions are reached, inoculation process was done on two 500 µL Plate Count Agars in each dilution, in order to count the number of colonies in one ml, and incubated for 72 hours at 30°C. After the incubation, the colonies in petri dishes that were inoculated in the dilution factor of 10^{-6} were counted, (500 µL + 500 µL) in one ml in total.

IV. MATERIALS & METHOD

The experiment was conducted in Ankara Food Control Laboratory Directorate's Microbiology Lab with Mrs Arzu Birinci who works there as a laborant.

Material List

- 18x150 mm test tube
- Ringer tablet
- Plate Count Agar and Skim Milk Agar
- 1000 ml glass schott bottle
- 100x15 mm glass petri dish
- Distilled water
- 50 ml plastic centrifuge tube
- 10 ml glass stripette
- Pipette controller
- 0.1M HCl solution
- Glass florence flask
- Drigalski spatula
- Micropipette
- Black boardmarker
- Vortex
- Autoclav

Ringer Solution Preparation

1. Prepare a ringer solution by stirring 500 ml of distilled water with a tablet of ringer at 100°C in magnetic stirrer.
2. Fill 50 of the test tubes with 9 ml of prepared ringer solution. Sterilize them by autoclaving for 15 minutes at 121°C.

Preparation of Agar Plates

1. Weigh 17.5 gram of Plate Count Agar and one gram of Skim Milk Powder by using scale and put them into 1000 ml glass schott bottle.
2. Add one liter of distilled water to schott bottle to prepare one liter of medium, and sterilize at 121°C for 15 minutes by autoclaving.
3. Pour the autoclaved medium to the 20 ml of the petri dishes after the mediums cooled to 45°C.

Preparation of Ringer Dilutions

1. Mix the prepared 9 ml of ringer solution with one ml of kefir sample in a test tube by using micropipette. Then, use the vortex for five seconds to homogenize the combination. This has a dilution factor of 10^{-1}.
2. Take one ml of 10^{-1} dilution from its test tube by using micropipette, and pour the one ml combination to the second test tube. Use the vortex for five seconds. This has a dilution factor of 10^{-2}. Repeat the same step until the dilution factor of 10^{-6} is reached.

Evaluation of Total Number of Colonies in Kefir Samples

1. When all the dilution factors are reached, take 100 µL from each sample and inoculate it to the agar plates respectively by using sterile drigalski spatula.
2. Incubate the inoculated agar plates at 30°C for 72 hours.
3. Perform three trials.
4. Calculate and use the mean values of the results.

Inoculation of kefir samples with HCl

1. Take five plastic 50 ml centrifuge tubes.
2. Eject 9 ml of Homemade kefir sample from its bottle by using the pipette controller, pour it into the first centrifuge tube.

3. Eject 9 ml of Brand one kefir sample from its bottle; pour it into the second centrifuge tube. Repeat this process for the other kefir samples.
4. Pour one ml of 0.1M HCl into the first centrifuge tube.
5. Fill each of the remaining four of the centrifuge tubes with one ml of 0.1M HCl.
6. Incubate all five of the centrifuge tubes for four hours at 37°C.
7. Take out the centrifuge tubes from the incubator after four hours.
8. Mix one ml of the acidified kefir sample with 9 ml of ringer solution in a test tube. This has a dilution factor of 10^{-1}. Repeat this step until the dilution factor of 10^{-6} is reached.
9. When all the dilution factors are reached, take 500 µL from the test tube, then inoculate to the petri dishes. Repeat this step for the remaining four kefir samples.
10. Incubate all 30 of the petri dishes for 72 hours at 30°C.
11. Perform five trials.

Figure 2: Inoculated Kefir Samples on Agar Plates

V. DATA ANALYSIS

Type of Kefir		Mean Number of Colonies Reproduced on Agar
Homemade		292
Store Products	AOÇ	132
	Altınkılıç	116
	Ü.İçim	300
	Eker	44

Table 1: Table showing the mean number of colonies counted in 100 µL of homemade and fabrication kefir samples, which have a dilution factor of 10^{-6}, before being exposed to hydrochloric acid according to three trials. The samples (9 ml kefir) were diluted by the factor of 6, from these samples 100 µL of each spread onto agar plates.

Brand of Kefir	Number of Trials	Number of Colonies
Homemade	1	316
	2	284
	3	278
	4	300
	5	306
Ülker İçim	1	330
	2	296
	3	312
	4	324
	5	304
AOÇ	1	116
	2	108
	3	112
	4	132
	5	126
Altınkılıç (Strawberry Flavored)	1	160
	2	152
	3	124
	4	156
	5	128
Eker	1	28
	2	66
	3	50
	4	56
	5	48

Table 2: Table showing the counted number of colonies in 500 µL of acidified kefir samples, which have a dilution factor of 10^{-6}. The samples (9 ml kefir) were first acidified with 0.1M HCl and diluted by the factor of 6. From these samples, 500 µL of each spread onto agar plates.

Type of Kefir	Mean Number of Colonies
Homemade	296.8
Ülker İçim	313.2
Altınkılıç	144
AOÇ	118.8
Eker	49.6

Table 3: Table showing the mean number of counted alive colonies in 500 µL of acidified kefir samples, which have a dilution factor of 10^{-6}. The samples (9 ml kefir) were first acidified with 0.1M HCl and diluted by the factor of 6. From these samples, 500 µL of each spread onto agar plates.

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Statistical Analysis

1. Mean

$$\mu = \frac{1}{n}\sum_{i=1}^{n} x_i$$

n: number of trials

x: number of alive bacteria

$\frac{316+284+278+300+306}{5}$ = 296.8

2. Standard Deviation

$$\sigma = \sqrt{\frac{\sum (x-\bar{x})^2}{n}}$$

σ = standard deviation

$\sum$ = sum of

x = each value in the data set

$\bar{x}$ = mean of all values in the data set

n = number of value in the data set

$$\sqrt{\frac{[(316-296.8)+(284-296.8)+(278-296.8)+(300-296.8)+(306+296.8)]}{5}}$$

$= \sqrt{798.848} = 28.3$

3. Standard Error

SE = $\frac{s}{\sqrt{n}}$ = $\frac{28.3}{\sqrt{5}}$ = 12.7

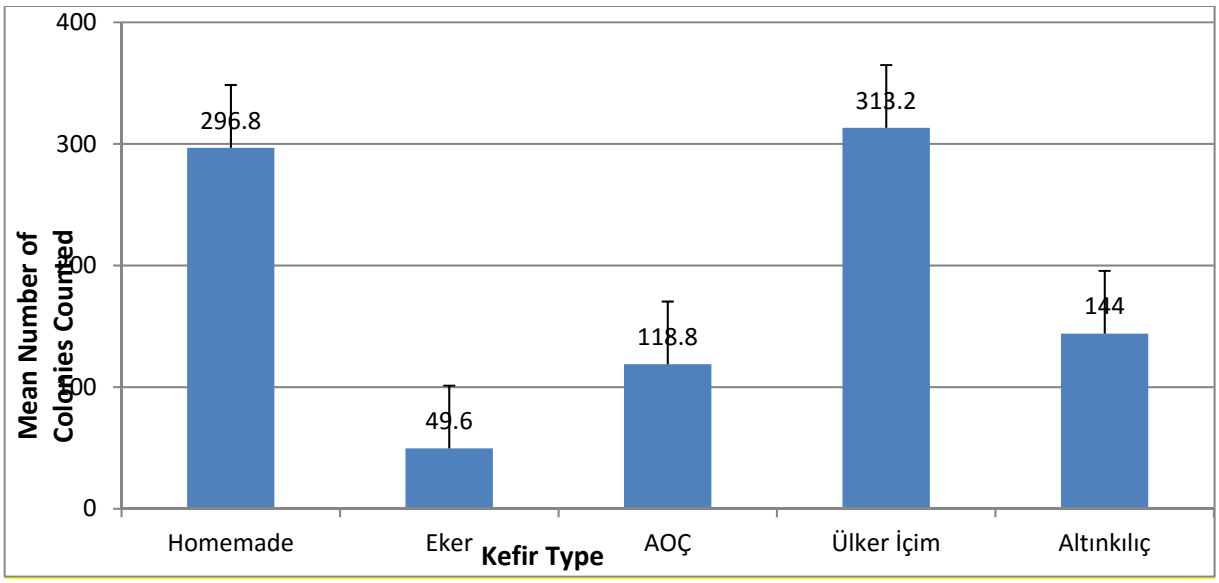
4. Variance

Variance = (standard deviation)^2

$(28.3)^2 = 801.0$

Type of Kefir	Mean	Standard Deviation	Variance (%)	Standard Error
Homemade	296.8	28.3	8.1	12.7
Ülker İçim	313.2	24.7	6.1	11.0
AOÇ	118.8	18.2	3.3	8.1
Altınkılıç	144	32.2	10.4	14.4
Eker	49.6	20.7	4.3	9.3

Table 4: The mean, standard deviation, variance and standard error values which were calculated by using number of the counted colonies as shown in Table 2.



Graph 1: Graph showing the comparison of mean number of colonies counted in 9 ml of each kefir sample exposed to one ml of gastric acid.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	266648,6	4	66662,16	328,5793	6,21E-18	2,866081
Within Groups	4057,6	20	202,88			
Total	270706,2	24				

Table 5: The ANOVA test results calculated from the data in Table 2.

VI. EVALUATION

The aim of this experiment is to investigate the total number bacteria colonies that resist stomach acid in homemade kefir and the four different brands (**Ülker İçim, Altınkılıç, AOÇ, Eker**) of kefir in a created, synthetic stomach environment. In order to fulfil the aim, 9 ml of each kefir sample was inoculated with HCl to a medium made up of Skim Milk Powder and Plate Count Agar, and incubated for 72 hours at 30°C, the results were shown in Table 2. It was hypothesized that "There is a considerable difference between the number of alive bacteria in homemade kefir and the four different brands of kefir bought from the supermarket after being exposed to stomach acid, known as hydrochloric acid. Homemade kefir will have the highest number of alive bacteria that resist stomach acid, while the other kefir types which are sold in the supermarkets will have lesser number of alive bacteria." Before conducting the main trials, a control trial was conducted to determine the number of alive bacteria

and the number of colonies (has a dilution factor of 10^{-6}) present in each kefir sample. Hydrochloric acid (gastric acid) wasn't used in order to figure out the reproduction capability of the bacteria under normal conditions. As it is seen in Table 1, the results show that **Ülker İçim** has the highest mean number of alive colonies, 300. **Ülker İçim** is unexpectedly followed by homemade kefir with 292 alive colonies. Homemade kefir is followed by **AOÇ** with 132 alive colonies and **Altınkılıç** (strawberry flavoured) has 116 alive colonies. There is a dramatic decrease in the number of alive colonies in **Eker**, it has the least mean number of colonies among others, 44.

According to the results of the main trials, in which hydrochloric acid was used, **Ülker İçim** showed the greatest similarity to homemade kefir with the mean number of alive colonies, 313.2. Meanwhile, the mean number of alive colonies present in the the homemade kefir was 296.8, which was hypothesized to have the highest number of alive bacterium. The homemade kefir was followed by **Altınkılıç** (strawberry flavoured) with the mean number of colonies, 144. **AOÇ** was in the fourth rank with the mean number of colonies, 118.8. The last kefir brand **Eker**, showed the slightest similarity to homemade kefir with the mean number of colonies, 49.6.

During the examination and comparison of the standard deviation data, **Altınkılıç** has the highest standard deviation among others, 32.2, which directly points out that it has the least reliance. After **Altınkılıç**, homemade kefir's standard deviation is 28.3 and it is followed by **Ülker İçim** which has a standard deviation of 24.7. **Eker** and **AOÇ** have closer standard deviations, **Eker** has 20.7 and **AOÇ** has 18.2, which means that **AOÇ** has the most reliance. When the results of ANOVA test is to be considered, the p-value is smaller than 0.05, which shows a significant mean difference between the number of colonies in kefir samples, thus the null hypothesis is rejected.

While conducting the experiment, there may have been some errors that influence the accuracy of the results.

- The experiment was performed in a synthetic stomach environment, and no enzymes were used. Therefore, no actual stomach environment was created for the bacterium to reproduce. Without the presence of the stomach and

pancreatic enzymes, a real reproduction activity may not occur and the results may be deceptive.

- While fermenting the homemade kefir, the optimum temperature, *between 20-25°C,* may have not obtained. Thus, the fermentation process may have not been completed. A half-fermented kefir may not provide accurate results.
- While fermenting the homemade kefir, a complete sterilization of grains which were kept in the refrigerator may have not attained since it is not as sterile as a lab environment. So, any other bacteria from home kitchen may have contaminated the kefir.
- The expiry dates of each kefir brand were the same, but through the transportation of the kefir packages there may have been differences.

VII. CONCLUSION

In my study, I made an investigation of the research question “Will there be a significant difference between the number of alive bacteria in homemade kefir and the four different brands of kefir bought from the store, with regard to their probiotic effects after incubating the cell suspensions from each sample at pH 2 by using Cell Viability Assay method?”

In the frame of this research question, as stated in my hypothesis “There is a considerable difference between the number of alive bacteria in homemade kefir and the four different brands of kefir bought from the supermarket after being exposed to stomach acid, known as hydrochloric acid. Homemade kefir will have the highest number of alive bacteria that resist stomach acid, while the other kefir types which are sold in the supermarkets will have lesser number of alive bacteria.” After obtaining the final results concerning the number of alive probiotic bacteria counted in the kefir samples, it was observed that the sample of **Ülker İçim** contained the highest number of alive probiotic bacteria after being exposed to hydrochloric acid. It was seen that the homemade kefir sample was containing the second high number of alive probiotic bacteria. There was a slight difference between the homemade kefir and **Ülker İçim** samples regarding the number of alive probiotic bacteria. There was another unexpected result showing that **Eker** which was generally consumed in our

house had the least number of alive probiotic bacteria. Hence, these results obtained didn't support my hypothesis. However, this investigation could be conducted on a created stomach environment with enzymes and developed using a wide range of kefir brands with a systematic review of trials. But this way of experimentation was above my capacity.

In order to obtain more accurate and precise results, there are a few things that could have done before and during the experiment.

- The kefirs should have been directly bought from the manufacturer. Thus, there wouldn't be any differences during the transportation of the bottles.
- Methylene Blue Counting method should have been used instead of Cell Viability Assay. It has less limitation than the other methods as it shows real living colonies and helps having more accurate results.
- The number of alive bacteria can also be counted with spectrophotometry.
- During the experiment, only the number of colonies was counted, but species of the bacterium should also be defined.
- Aside with the gastric acid, a synthetic duodenum environment with gastric enzymes should have been created. Thus, it would be possible to figure out the most appropriate kefir sample in which the probiotic effect worked best.
- Other culture media can be used which supports the growth of bacteria such as M 17 and MRS media.
- All the cultures were incubated under aerobic conditions. Anaerobic culture chambers can be used to grow anaerobic bacteria.

My study provided a clear answer to the question about the benefits of kefir and could be explanatory to the ones who need to be informed about probiotic drinks and probiotic food. My investigation shows that both the home made and the commercially produced kefir brands comparatively contain probiotic alive bacteria that resist stomach acid and which is crucially important for human's health. Consequently kefir is a notably healthful drink and must be consumed regularly by all the people. In conclusion, the results of the experiment rejected the hypothesis which was formed as "Homemade kefir will have the highest number of alive bacteria that resist stomach acid, while the other kefir types which are sold in the supermarkets will have lesser number of alive bacteria."

BIBLIOGRAPHY

1. Irigoyen, A. et al. "Microbiological, physicochemical, and sensory characteristics of kefir during storage." Food Chemistry 90,4 (2005): 613-620.

2. Lee, Y. Salminen, S “Trends in Food Science and Technology” Coming of age of probiotics. (1995): 241-245(5).

3. Farnworth R Edward, “Kefir – a Complex Probiotic” Food Science and Technology Bulletin.

4. Author’s name not stated. Time and date not stated.

Kefir Health Benefits

http://www.regenerate-wellness.com/kefir.html

5..Ofiyeva, M. (2007) “Kefir.”

http://www.dermaharmony.com/ingredients/kefir.aspx

6.Riss, L Terry, PhD. Moravec, A Richard, BS. Niles, L Andrew MS. Benink, A Helene, PhD. Worzella, J Tracy, MS. Minor, Lisa, PhD. Storts, Douglas, PhD. Reid, Yvonne, PhD. (2013) “Cell Viability Assays”

7. Author’s name not stated. Time and date not stated.

http://www.oxoid.com/UK/blue/prod_detail/

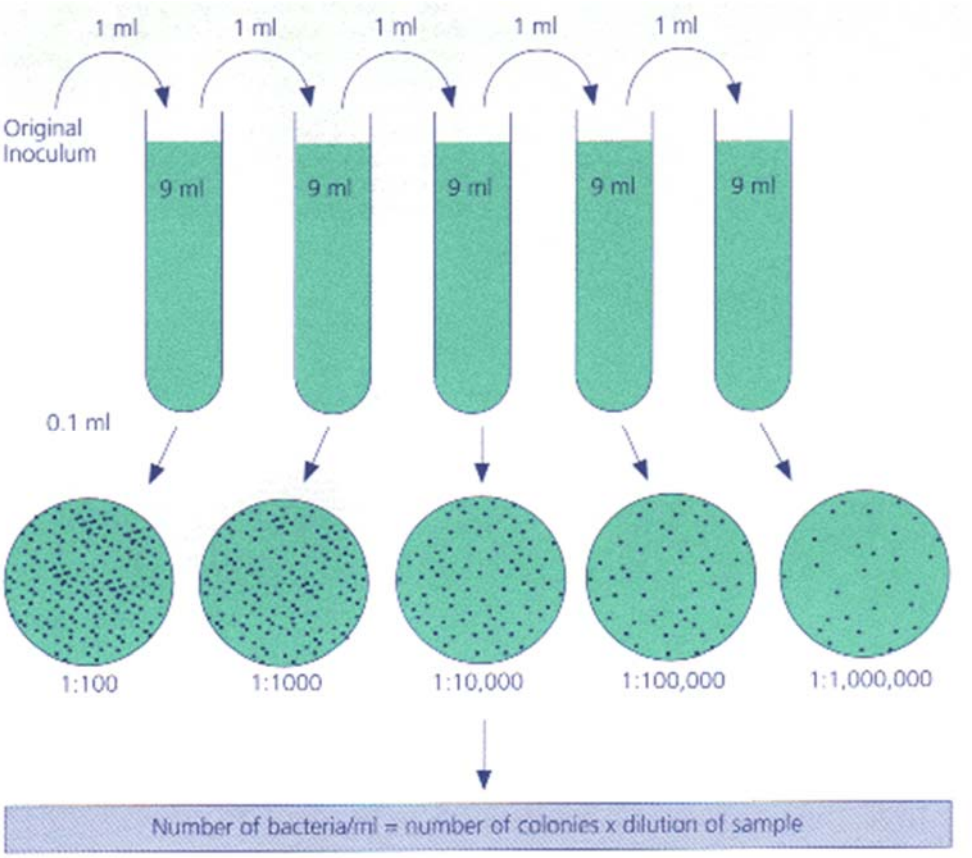
8. Author’s name not stated. Time and date not stated.

http://himedialabs.com/td/m763.pdf

Appendices

Appendix 1

Preparation of ringer serial dilutions



http://www.sigmaaldrich.com/analytical-chromatography/microbiology/learning-center/theory/introduction.html

Appendix 2

The Microflora of Kefir Grains

The Microflora of Kefir Grains

	Species
Yeasts	*Candida kefir*
	Candida pseudotropicalis
	Kluyveromyces marxianus subsp. *marxianus*
	Saccharomyces kefir
	Saccharomyces turicensis
	Torula spp.
	Other Yeasts
Bacteria	*Acetobacter aceti*
	Lactobacillus casei
	Lactobacillus helveticus
	Lactobacillus kefir
	Lactobacillus kefirogranum
	Lactobacillus kefironafaciens
	Lactococcus lactis subsp. *lactis*
	Leuconostoc mesenteroides subsp. *dextranicum*
	Leuconostoc mesenteroides subsp. *cremoris*

Mistry, Vikram V. "Fermented liquid milk products." Handbook of Food Science, Technology, and Engineering (2006).