TED ANKARA COLLEGE FOUNDATION HIGH SCHOOL

Comparison of the antibacterial effects of different fermented milk products; yoghurt, probiotic yoghurt and kefir on *E.coli.*

Biology Extended Essay

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Word Count:

Abstract

The aim of this essay was to investigate the antibacterial effects of different fermented milk products (yoghurt, probiotic yoghurt and kefir) and compare their antibacterial effects on *E.coli.*

My research question was "Do commercial yoghurt, probiotic yoghurt and kefir have antibacterial effect and which of these products has the best antibacterial effect on *Escherichia coli*?

My hypothesis was that; there would be a significant difference in the means of bacteria growth after mixing with the products and probiotic yoghurt would have the best effect. To test the research question and the hypothesis, experiments were conducted. The method used was counting the numbers of colonies that grow on agar media. For this, bacterial suspension containing *E.coli* was mixed with each test substance separately and diluted. Then these suspensions were inoculated on the agar and the numbers of colonies grown on agar were counted.

The results of the experiment was analyzed with the appropriate statistical tests (descriptive statistics and ANOVA), it was seen that there was a significant difference between the means of the bacteria grown for different products, however, the hypothesis was rejected and yoghurt was accepted as the product which has the best antibacterial effect on *E.coli* followed by probiotic yoghurt and kefir.

(Word Count: 203)

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I. Introduction

The first time I thought about focusing on this subject in my extended essay was the day I was watching the advertisements on the TV and came across an advertisement about the benefits of the probiotic yoghurt. It was indicated in this advertisement that probiotic yoghurt was more effective than normal yoghurt in terms of antibacterial action. I wondered if milk products can be effective on harmful bacteria especially the ones in the gastrointestinal tract flora because I know from the lectures that gastrointestinal diseases are the major health problems in Turkey. It attracted my attention so I made a research on internet and found out that yoghurt, kephir and probiotic yoghurt have some antibacterial effect.(1) So I decided to work on this subject to investigate and compare the antibacterial effects of yoghurt, probiotic yoghurt and kephir.

Yoghurt, kephir and probiotic yoghurt are produced by bacterial fermentation of milk. Yoghurt is produced by fermentation of milk by a defined microflora which consists of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* which are called as "starter" microorganisms. These bacteria are not pathogens, in contrast they are beneficial. They carry out lactose fermentation and produce lactic acid which acts on milk protein and give yoghurt its texture and flavour. The final product contains 1.2-1.4% lactic acid (2). "Yoghurt is nutritionally rich in protein, calcium, riboflavin, vitamin B6 and vitamin B12".(2) It has nutritional benefits beyond those of milk.

Kefir is produced traditionally or commercially in many parts of the world by adding kefir grains (starters) to pasteurized milk. "Kefir grains contain lactic acid bacteria, yeasts like *Saccharomyces* species and streptococci, namely *Leuconostoc cremoris* and *Lactococcus lactis*. During fermentation lactose forming yeasts produce alcohol and CO_2 and lactic acid bacteria convert lactose to lactic acid."(3)

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1. The Harvard Medical School Family Health Guide. "Health benefits of taking probiotics". Harvard University, 2005. Web. 18.12.2011 <http://www.health.harvard.edu/fhg/updates/update0905c.shtml >
2. Robinson, Richard K., Carl A. Batt, Pradip D. Patel. Encyclopedia of Food Microbiology, Vol. 2, Academic Press 2000, London, UK
3. Montville, Thomas J, Mathews, Karl R. Food Microbiology. ASM Press, 2nd. Ed. 2008, USA

Probiotics which are added to certain foods, are defined as "a live microbial feed supplement which beneficially affects the host organism by improving its intestinal microbial balance"[4] Today, many dairy products that contain probiotics are on the market since their effect to human nutrition has been increasingly recognized. "The most common genus found in probiotics supplements tend to be Lactobacillus and Bifidobacterium species"[5]. Lactic acid bacteria (LAB) like *Lactobacillus acidophilus* and bifidobacteria like *Bifidobacterium bifidus* are the most common types of "friendly bacteria" used as probiotics; but certain yeasts such as *Saccharomyces boulardii* and also used. Probiotics are commonly consumed as part of fermented foods with specially added active live cultures; such as in yogurt, soy yogurt, or as dietary supplements [6].

There are many "friendly" microorganisms which help to keep the balance in the intestines and "unfriendly" microorganisms such as bacteria, yeasts, fungi, and parasites in the normal flora of the gastrointestinal tract. Most probiotics are bacteria similar to those naturally found in people's intestines. All of these bacteria in normal flora help to maintain certain nutritional and digestive benefits, inhibition of colonization and infection by pathogenic microbes like *E.coli* and possibly more pathogenic bacteria. They produce lactic acid, ammonia, hydrogen peroxide and bacteriocins[6].

I know from the lectures that diarrhea caused by the harmful bacteria is one of the major gastrointestinal diseases in Turkey. I made a research on the internet to learn what exactly cause diarrhea and what diarrhea is so, "Diarrhea is an increase in the frequency of bowel movements or a decrease in the form of stools (greater looseness of stool)"[7] Diarrhea is generally caused by bacteria and *Escherichia coli* is one of the most frequent agents of diarrhea. *E. coli* are among the normal members of gastrointestinal flora and are usually harmless. However, if the number of these bacteria increase or their location changes they may cause some diseases like diarrhea.[8]

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4. Montville, Thomas J, Mathews, Karl R. Food Microbiology. ASM Press, 2nd. Ed. 2008, USA
5. "Bacterial, Fungal & Parasitic Overgrowth." Web. 18.12.2011 <http://www.medicalinsider.com/bacterial.html#probioticsgeneral>
6. Alvarez-Olmos Martha I, Oberhelman Richard A. Probiotic Agents and Infectious Diseases: A Modern perspective on a Traditional Therapy. Clinical Infectious Diseases 2001, 32: 1567-1576
7. "What is diarrhea" <www.medicinenet.com/diarrhea/article.htm> Web. 21.08.2011
8. Todar, Kenneth. Todar's Online Textbook of Bacteriology. <http: //www.textbookofbacteriology.net> Web 18.12.2011

The above information led me think that these fermented milk products may have antibacterial activity thus inhibiting the growth of pathogenic intestinal bacteria such as diarrhea causing bacteria, like *E.coli* and act similar to antimicrobial agents. If this is proved, these products may be used as an alternative to antibiotics to inhibit bacterial growth and can be used to help curing diarrhea.

The reason I chose *E.coli* as the test bacteria in my experiments was that it was a very common type of bacteria. They also grow very rapidly, easy to obtain and widely used in the experiments. Most importantly it is one of the bacteria which may cause gastrointestinal tract diseases[(9)].

Many people describe these fermented milk products in their reports as helpful for stomach and digestive upset, for intestinal gas, and even for inflammatory problems of the gastrointestinal tract, but these reports are more anecdotal than proved by research. There are also some papers discussing the antibacterial effects of these products; however I didn't come across a paper comparing those three products [(10, 11, 12)]. So this is the reason I decided to compare yoghurt, probiotic yoghurt and kefir's antibacterial effect.

So this investigation was aimed to determine whether these fermented milk products exert antibacterial activity against *Escherichia coli* and which of them have the best antibacterial activity. Consequently, this paper will focus on the research question: "Do commercial yoghurt, probiotic yoghurt and kefir have antibacterial effect and which of these products has the best effect on *Escherichia coli* in terms of bacteria growth which was measured by colony counting method?

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9. Todar, Kenneth. Todar's Online Textbook of Bacteriology. http: //www.textbookofbacteriology.net on 18.12.2011
10. Kotz, Catherine M, Peterson, Lance R, Moody, Julia, A, Savaiano, Dennis A, Levitt, Michael D. In vitro antibacterial effect of yogurt on Escherichia coli. Digestive Diseases and Sciences 1990, 35: 630-637.
11. Silva KR, Rodrigues SA, Filho LX, Lima AS. Antimicrobial activity of broth fermented with kefir grains. Applied Biochemistry and Biotechnology 2009, 152: 316-325
12. Chuayana Jr. Eduardo L, Ponce Carmina V, Rivera Ma. Rosanna B, Cabrera Esperanza C. "Antimicrobial activity of probiotics from milk products." Philippine Journal of Microbiology and Infectious Diseases 2003. Web 12.08.2011 <http://www.psmid.org.ph/vol32/vol32num2topic3.pdf>

II. Hypothesis

Fermented milk products like yoghurt, kefir and probiotics help to maintain the balance in gastrointestinal tract. Yoghurt results from the fermentation of the milk by *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus.* Kefir is produced by the kefir grains containing *Saccharomyces* species , *Leuconostoc cremoris* and *Lactococcus lactis*. Probiotic yoghurt is produced by adding the probiotic bacteria such as lactic acid bacteria (LAB) like *Lactobacillus acidophilus* and bifidobacteria like *Bifidobacterium bifidus* [13].

Infectious diarrhea is one of the most common infectious diseases and *E. coli* is a frequent cause of diarrhea. Many children lose their lives due to diarrhea and antibiotics are usually not recommended for treatment of diarrhea [14, 15]. If the antibacterial effects of these milk products are proved by this study, they can be used in the establishment and continuity of gastrointestinal tract "friendly" flora and prevent diarrhea.

In the light of this information, I expect that there will be a significant difference between the effectiveness of these fermented milk products on *E.coli* since the bacteria in these fermented milk products are different. It is predicted that probiotic yoghurt will have the better antibacterial effect on *E. coli* because probiotic yoghurts contain more friendly bacteria than the others. So my hypothesis is that probiotic yoghurt will be the most effective product against *E. coli* followed by kefir and then yoghurt.

13. Robinson, Richard K., Carl A. Batt, Pradip D. Patel. Encyclopedia of Food Microbiology, Vol. 2, Academic Press 2000, London, UK
14. Todar, Kenneth. Todar's Online Textbook of Bacteriology. <http: //www.textbookofbacteriology.net> on 18.12.2011
15. "What is diarrhea" <www.medicinenet.com/diarrhea/article.htm> Web. 21.08.2011

III. Method Development

My research question in this experiment is "Do yoghurt, probiotic yoghurt or kefir have antibacterial effect on *E.coli* and which one of them have the best effect?" To test this research question and to prove or disprove the hypothesis, data must be obtained and compared with statistical tests.

I decided to work with three fermented milk products since they are the most common ones consumed in Turkey. Yoghurt and probiotic yoghurt were bought from the market but kefir was prepared by me since people generally do not prefer to use commercial kefirs. (16)

The next step was deciding on the microorganism to be used in the experiment and obtaining it. *E.coli* was chosen to be the test bacteria since it is the most common gastrointestinal disease causing bacteria. The test bacteria used in this experiment were *Escherichia coli* ATCC 25922 since it is the standard *E.coli* found in the gastrointestinal tract. These bacteria were obtained from Hacettepe University Medical Faculty Department of Clinical Microbiology.

Then, a method was designed for testing and comparing the three substances' antibacterial activity. I searched on the internet and came across a method called zone of inhibition test which is widely used for testing the antibacterial activity of antibiotics (17). I planned to measure the inhibition zones around the paper discs containing test substances. For this, a portion of each test substance was taken into sterile test tubes and filtrated. Standard paper discs (6 mm diameter) were soaked with the test substances and let air dried. Also commercially prepared ceftriaxone discs were used as the control antibacterial agent. Paper discs containing test substances and the control antibiotic were placed onto Mueller-Hinton agar inoculated with bacteria (18).

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16. E-notes. "Kefir". Web. 18.12.11. http://www.enotes.com/topic/Kefir

17. Antimicrobial Test Laboratories. "Zone of inhibition test". Web. 31.01.2011 <www.**antimicrobialtestlaboratories**.com/Zone.... >

18. Hudzicki, Jan. "Kirby-Bauer Disk Diffusion Susceptibility Test Protocol". 08 December 2009. American Society of Microbiology. Web. 18.12.2011. <http://www.microbelibrary.org/component/resource/laboratory-test/3189-kirby-bauer-disk-diffusion-susceptibility-test-protocol>

However, this method did not work; no inhibition zones developed around the discs after 24 h. incubation. The problem of this method might be insufficient absorbance of paper discs or insufficient diffusion of the test substances from the paper discs into the agar medium. I repeated this method twice and since I obtained the same result I decided to perform another method for testing the antibacterial activity.

I searched on the internet to find a better method to test the antibacterial effect of the milk products that can also mimic the gastrointestinal tract flora and came up with this method which I learned from the articles. This method was counting the numbers of colonies that grow on agar media [19]. For this bacterial suspensions were prepared and turbidity was adjusted.[20] The bacterial suspension containing *E.coli* was mixed with each test substance separately. Since the number of the colonies might be too much to count after inoculation of these suspensions onto agar media, I decided to dilute the suspension in test tubes before inoculating them.

After dilution 10 µl of each mixture with each dilution factor was inoculated onto Mac Conkey agar. This agar was used in this experiment so that other types of bacteria that can be present in test substances cannot grow and only *E.coli* can grow [21].

Each test was repeated for five times for each test substance. All of the test plates were placed into the incubator and incubated at 37°C for 24 hours. Next day number of colonies on each agar plate were counted and recorded. Then number of colonies recorded was multiplied by the dilution factors.

The reason I decided to use this method was that it was used frequently in similar investigations and this method allowed me to collect data which I could use in the statistical tests to compare the antibacterial activity of the test substances.

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19. Martinez-Costa C, Silvestre MD, Lopez MC, Plaza A, Miranda M, Guijjaro R. Effects of refrigeration on the bactericidal activity of human milk: A preliminary study. Journal of Pediatric gastroenterology and Nutrition 2007, 45: 275-277.
20. "McFarland Standards". <http://www.bd.com/ds/productCenter/245015.asp> Web. on 8.10.2011
21. Allen, Mary E. "MacConkey Agar Plates Protocols". 30 September 2005. American Society of Microbiology. Web. 18.12.2011..<http://www.microbelibrary.org/component/resource/laboratory-test/2855-macconkey-agar-plates-protocols>

Materials:

Test substances:

- Commercial yoghurt
- Probiotic yoghurt
- Kefir

Test bacteria:

- *Escherichia coli*

Materials to be used in the experiment:

- Sterile physiological saline solution (0.9% NaCl)
- Sterile disposable plastic syringe (10 ml)
- Automatic pipette (100-1000 µl)
- Automatic pipette (10-200 µl)
- Plastic sterile petri dishes x 20 (9 cm diameter)
- Sterile glass test tubes x 25 (10.0 ml)
- Wire loop x 2
- Blood agar medium
- Brain Heart Infusion Broth (BHIB)
- MacConkey agar medium

Instruments:

- Bunsen burner
- Vortex (mixer)
- Shaking incubator
- Incubator

IV. Method

Mask, gloves and laboratory coat were worn during the experiment to prevent contamination of the test system by external factors.

A. Preparation of the test substances

1. Yoghurt and probiotic yoghurt were bought commercially.
2. Kefir was produced by boiling 500 ml pasteurized daily milk, cooling it down to 25°C and adding 10g kefir colonies.
3. It was kept at 20-25°C for 24 hours and put in the refrigerator for another 24 hours.
4. The liquid part was filtered through a strainer.

B. Preparation of the test bacteria

1. The bacteria kept at -80°C were first liquified at room temperature.
2. 20 µl of *E.coli* suspension were inoculated onto blood agar media and spread on the agar by a sterile wire loop.
3. The plates were placed in a 37°C incubator and incubated for 24 hours.

C. Conducting the experiment

1. Colonies of the test bacteria were transferred from blood agar media into Brain Heart Infusion Broth (BHIB) and incubated at 37°C for 24 hours.
2. One loopful of bacteria from this suspension was taken into 2 ml sterile saline solution and turbidity of the bacteria was adjusted to 1 McFarland Standard(see Appendix 1)
3. 4.5 ml of each filtrated test substances were placed into the glass tubes by the help of an automatic pipette and 0.5 ml of *E.coli* was added to the tubes. 4.5 ml of BHIB and 0.5 ml of *E.coli* was also mixed in a tube as the control. The tubes were mixed by the vortex and placed in a shaking incubator and incubated at 37^{o}C for 2 hours. Shaking helped better growth of bacteria and also more homogeneous interaction of the test substance and bacteria.

4. The mixture was diluted for 4 times

The serial dilution was conducted as in the figure:

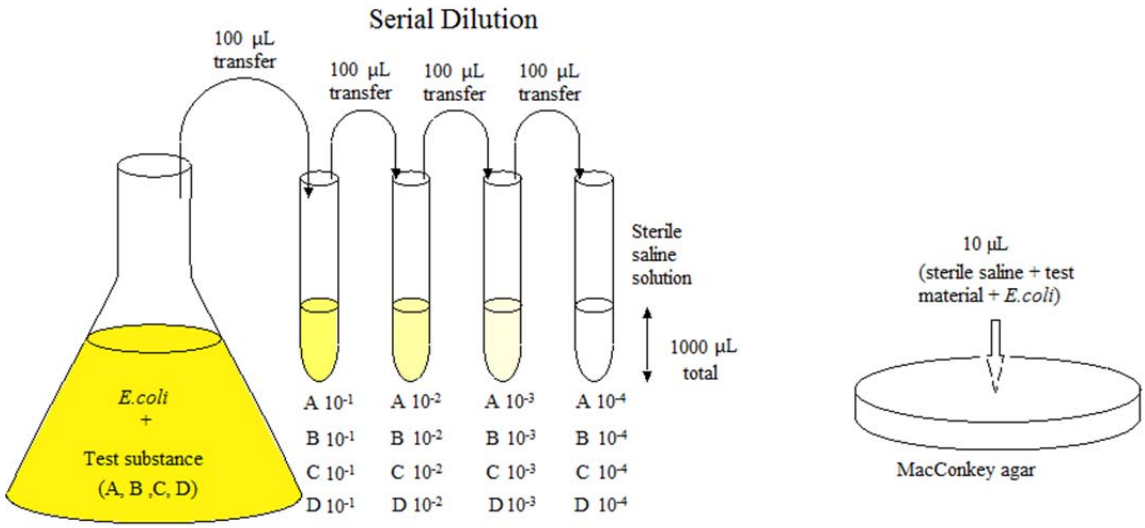


Figure 1: The serial dilution method used to dilute the mixture of E.coli and the test substance. A represents yoghurt, B represents probiotic yoghurt, C represents kefir and D represents the control group.

5. At the end of the serial dilution process, 10 µl of mixture was taken from the test tube "A" 10^{-1} and inoculated onto the surface of Mac Conkey agar as in figure 1. The mixture was spread on the agar with a wire loop that has been sterilized by the bunsen burner and the petri dish was closed quickly to prevent contamination. This was repeated for all dilutions and for all test substances.

D. Antibacterial activity testing by colony count method

1. The number of colonies on each agar plate were counted by hand.
2. Number of colonies recorded was multiplied by the dilution factors.

E. Data analysis

1. The means of the numbers of the colonies grown were calculated

2. Microsoft Office Excel 2003 was used to calculate count, sum, average and variance values
3. ANOVA (Analysis of Variance) test was done to see if there is a significant difference between the means
4. Independent T-test was used to see the mean difference of pairs.
5. Descriptive statistics which are standard deviation, standard error and confidence interval were calculated

V. Results

Results obtained by the data collected are shown in Table 1 and Table 2 below.

Table 1: Raw data showing the number of colonies grown on agar for different test groups and different dilution factors after 24 hours.

Test Materials	Trials / Dilution	Number of colonies				
		1	2	3	4	5
Yoghurt (A)	10^{-3}	0	0	0	0	0
	10^{-4}	0	12	22	4	45
Probiotic Yoghurt (B)	10^{-3}	110	148	170	200	200
	10^{-4}	19	60	66	68	72
Kefir (C)	10^{-3}	0	188	135	184	170
	10^{-4}	16	90	70	160	68
Control (D)	10^{-3}	860	400	580	640	800
	10^{-4}	625	180	170	175	260

VI. Data Analysis

The following formulas were used to calculate mean, standard deviation, standard error and confidence interval for descriptive statistics of the 3 test groups.[22]

1. Mean:

$$\bar{x} = \frac{1}{n} \cdot \sum_{i=1}^{n} x_i$$

n= number of trials

x_i= number of colonies grown for trial i.

2. Standard Deviation:

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \mu)^2}, \quad \text{where} \quad \mu = \frac{1}{N}\sum_{i=1}^{N} x_i.$$

n= number of trials

x_i= number of colonies grown for trial i.

3. Standard Error

$$SD_{\bar{x}} = \frac{\sigma}{\sqrt{n}}$$

σ= standard deviation

n= sample size

4. Confidence Interval

95% CI = SE x $t_{(n-1)}$

SE = Standard error , t = the value of t at p= 0.05

22. "Mean", "Standard Deviation", "Standard Error", "Confidence Interval". Wikipedia the Free Encyclopedia. Web. on 8.10.2011

Table 2: Means of the number of the colonies grown for different dilution factors on agar after 24 hours for different test groups.

Test Materials	Number of colonies (x 10^7)				
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
Yoghurt (A)	0	0.6	1.1	0.2	2.25
Probiotic Yoghurt (B)	1.50	3.74	4.15	4.40	4.60
Kefir (C)	0.8	5.44	4.18	8.92	4.25
Control (D)	35.55	11.00	11.40	11.95	17.00

Table 3: The results of the ANOVA (Analysis of Variance) test for the test groups.

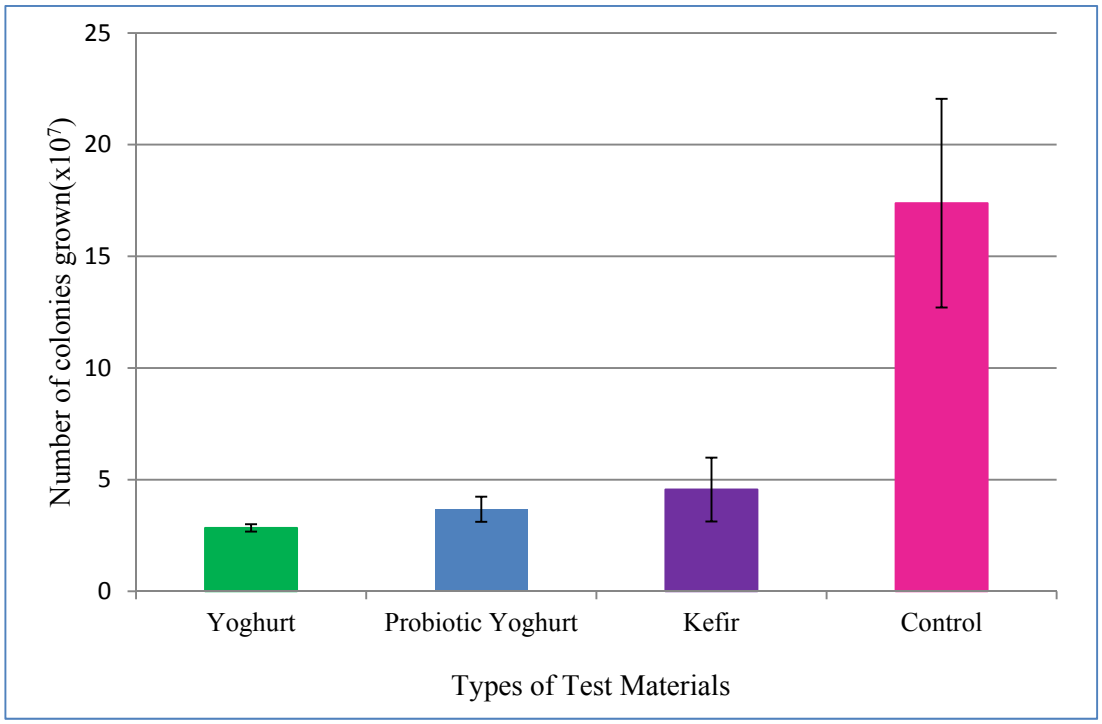
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	807,8535	3	269,2845	8,978957	0,001015	3,238872
Within Groups	479,85	16	29,99062			
Total	1287,703	19				

Table 4: Independent t-test (t-test: two sample assuming unequal variances) results used to compare the existence of significant difference between the test groups in pairs.

Matching Groups	P-value	Existence of significant difference (P<0.05)
Yoghurt vs. probiotic yoghurt	0.004475	Yes
Probiotic yoghurt vs. kefir	0,497060	No
Yoghurt vs. kefir	0.035894	Yes

Table 5: Descriptive statistics including mean, standard error, standard deviation and confidence interval for yoghurt, kefir, probiotic and control group.

	Count	Mean	Standard Error	Standard Deviation	Confidence Interval (95.0%)
Yoghurt	5	0.83	0.401746	0.898332	1.115426
Probiotic Yoghurt	5	3.678	0.563102	1.259135	1.563422
Kefir	5	4.718	1.304555	2.917074	3.622026
Control	5	17.38	4.670348	10.44322	12.96696



Graph 1: The comparison of bactericidal effects of test materials against E.coli in terms of number of colonies grown on agar.

Standard Error values were used as the error bars since they are the smallest among standard error, standard deviation and confidence interval.

VII. Evaluation

The research question of this investigation was “Do commercial yoghurt, probiotic yoghurt and kefir have antibacterial effect on *Escherichia coli* and which of these products has the best effect”. So the aim of this study was to find out if there is a significant antibacterial effect of yoghurt, probiotic yoghurt and kefir on *E.coli*, and to compare their antibacterial effects. It was hypothesized that there will be a significant difference between the effectiveness of these fermented milk products on *E.coli*. It is predicted that probiotic yoghurt will have the better antibacterial effect on *E. coli* followed by kefir and then yoghurt.

The results of the experiment showed that all of the products had antibacterial effect on *E.coli*. Their activity were compared by using Student’s t-test and found out that there was a significant difference between their means for two pairs(Yoghurt-probiotic yoghurt and yoghurt-kefir) but there was not a significant difference between a pair(probiotic yoghurt-kefir) as indicated in table 4. When I compared the means of the three groups, as it was seen in table 5, yoghurt had the strongest antibacterial effect on *E.coli* followed by probiotic yoghurt and kefir. The means of the number of the colonies grown were 0.83, 3.678, and 4.718 respectively. So less bacteria grew in the mixture containing yoghurt.

My null hypothesis was that there was no significant difference between the effectiveness of yoghurt, probiotic yoghurt and kefir on *E.coli*. Three test groups and the control group were tested by ANOVA and the p-value calculated by ANOVA was found out to be 0,001946 which was smaller than 0.05 so my null hypothesis was rejected and my hypothesis “There is a significant difference between the effectiveness of yoghurt, probiotic yoghurt and kefir on *E.coli”* was accepted.

To comment on the reliability of the experiment descriptive statistics were used. The confidence interval values were small; however, as it is compared to standard error and standard deviation they were slightly big. So it shows that the experiment is reliable but the data are not 100% coherent. The standard deviation values are big according to means of the test groups so it shows that the numbers of the colonies grown were not distributed homogenously although it should have been so. Standard error results are smaller when they are compared to standard deviation and mean values. This shows that there were some errors in the experiment. However, while the experiment was being conducted, there wasn’t any

problem occurred which may cause random errors by the experimenter; but there might have been some systematic errors caused by the method of the experiment.

The problems that might have been occurred and the improvements to the method to lessen the errors during other repetitions of the experiment is as follows:

1. The consistency of the products: Since the fermented milk products were different they had different consistencies. To make a more controlled experiment, water can be added to yoghurt and probiotic yoghurt so that all of them have the same consistency.

2. Production and expiration dates: The production and expiration dates were important because they affect the acidity. So to make a more controlled experiment kefir can be bought commercially or yoghurt and probiotic can be produced at home.

3. The acidity of the products: The acidity of the products were measured by pH papers and it was found out that they all have the same pH but for further researches acidity should be measured by pH meter to reach more accurate results.

Limitations of this extended essay:

1. The brands of the yoghurt and probiotic yoghurt: Both of the brands of the yoghurt used was Danone, so the type of the preservatives were the same. However, the other brands of yoghurts was not included in the in this study so the extent of this extended essay is limited with only one brand.

2. The limitation of using one species of bacteria: Only one type of bacteria was used during the experiments to make the experiment a controlled experiment because for other bacteria the results may be different.

My prediction about probiotic yoghurt being the most effective antibacterial agent was rejected because yoghurt shows a better antibacterial effect as discussed above. The reasons for their different antibacterial effect and yoghurt being the best antibacterial agent may be as listed below:

1. The reason for yoghurt being the best can be the different metabolic activity of different bacteria present in the products. “Fermenting foods are typically very complex ecosystems with active enzyme systems from the ingredient materials

interacting with the metabolic activities of the fermentation organisms. Factors such as added salt, particle sizes, temperature, and oxygen levels will also have important effects on the chemistry that occurs during fermentation."[23] Thus, because of the enzyme systems, metabolic activities such lactic acid fermentation can be effected and the rate of fermentation in the products can be different which may also contribute to yoghurts best antibacterial effect.

2. The acidity of the products were the same when it was measured by pH paper; however, the acidity of the yoghurt might change during the experiment because in yoghurt the bacteria carry on with lactic acid fermentation. [24]

3. The preservatives added commercially might also contribute to this result because kefir was produced at home so there were not any preservatives added and normally yoghurt has a longer shelf life than probiotic yoghurt so there might be substances that prevent the growth of microorganisms in yoghurt which also affected the antibacterial effect of yoghurt.

23. MCFEETERS, R. F. (2004), Fermentation Microorganisms and Flavor Changes in Fermented Foods. Journal of Food Science, 69

24. Robinson, Richard K., Carl A. Batt, Pradip D. Patel. Encyclopedia of Food Microbiology, Vol. 2, Academic Press 2000, London, UK

VIII. Conclusion

My research question “Do commercial yoghurt, probiotic yoghurt and kefir have antibacterial effect and which of these products has the best effect on *Escherichia coli* ?” was answered with the use of appropriate statistical tests and it was found out that there is a significant difference between the antibacterial effect of yoghurt, probiotic yoghurt and kefir on *E.coli*. Yoghurt has the best antibacterial effect against *E.coli* followed by probiotic yoghurt and then kefir. Even though the prediction was rejected the study can be considered as successful because yoghurt being the best antibacterial agent is supported by the relevant arguments. The conditions which contributed to this result should be investigated in further researches so that the what exactly cause yoghurt having the best antibacterial effect can be explained .

The reason I chose to deal with this subject is that there were no other studies done to test the antibacterial effect of yoghurt, probiotic yoghurt and kefir at the same time. Other studies were separately done. Also, the reason I chose E.coli is that it is one of the bacteria that cause diarrhea which is a very common disease in Turkey. The importance of testing the antibacterial effect of these products is that to test if they can be used as an alternative to antibiotics. The results of the experment showed that these products have an antibacterial effect so they can be used to help curing gastrointestinal tract and also other diseases.

IX. Appendices

Appendix 1:

McFarland Standards

To estimate the organism densities in suspensions turbidity standards are used. This allows the visual comparison of organism densities. The most widely accepted standard is the McFarland standard. A McFarland standard is prepared by adding barium chloride to aqueous sulfuric acid. The density of the resulting barium sulfate precipitate can be used to estimate the number of colonies of a prepared suspension; e.g., In 1 McFarland there are approximately 3 x 10^8 CFU/mL." [25]

Appendix 2:

5% Sheep Blood Agar

	gm/litre
Proteose peptone	15.0
Liver digest	2.5
Yeast extract	5.0
Sodium chloride	5.0
Agar	12.0
pH 7.4 ± 0.2 @ 25°C	

Directions

"Suspend 40g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 45-50°C and add 5% sterile blood.

Mix with gentle rotation and pour into sterile petri dishes or other containers."[26]

25. "McFarland Standards". <http://www.bd.com/ds/productCenter/245015.asp> Web. on 8.10.2011

26. "5% Sheep Blood Agar". www.oxoid.com Web. on 8.10.2011

Description

"Oxoid Blood Agar Base No.2 was developed to meet the demand for an especially nutritious blood agar base which would permit the maximum recovery of delicate organisms without interfering with their haemolytic reactions"[26]

Appendix 3:

MacConkey Agar

"A differential medium for the isolation of coliforms and intestinal pathogens in water, dairy products and biological specimens."[26]

Typical Formula*	**gm/litre**
Peptone	20.0
Lactose	10.0
Bile salts	5.0
Sodium chloride	5.0
Neutral red	0.075
Agar	12.0
pH 7.4 ± 0.2	

Directions

"Suspend 52g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Pour into sterile petri dishes. Dry the surface of the gel before inoculation."[26]

Description

"A differential medium for the detection, isolation and enumeration of coliforms and intestinal pathogens in water, dairy products and biological specimens. Although principally used for coliforms, this medium may also be employed for the differentiation of other enteric bacteria (including pathogens)."[26]

Appendix 4:

Brain Hearth Infusion Broth

"A highly nutritious infusion medium recommended for the cultivation of bacteria including the fastidious organisms."[26]

Formula	**gm/litre**
Brain infusion solids	12.5
Beef heart infusion solids	5.0
Proteose peptone	10.0
Glucose	2.0
Sodium chloride	5.0
Disodium phosphate	2.5
pH 7.4 ± 0.2 @ 25°C	

Directions

"Dissolve 37g in 1 litre of distilled water. Mix well and distribute into final containers (tubes, bottles). Sterilize by autoclaving at 121°C for 15 minutes."[26]

Description

"A versatile liquid infusion medium which is suitable for the cultivation of many bacteria including the fastidious ones."[26]

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