TED ANKARA COLLEGE FOUNDATION HIGH SCHOOL

BIOLOGY EXTENDED ESSAY

Comparison of homemade yoghurt and manufactured yoghurts in terms of their pH change during shelf life as an indication of lactic acid production due to the presence of probiotic bacteria

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Abstract

The aim of this experiment is to show the differences between pH changes of homemade yoghurt and four different manufactured yoghurts by looking at their lactic acid production during shelf life which is an indication of increase in number of lactic acid bacteria.

My research question was *"Is there a significant mean difference between homemade and manufactured yoghurts in terms of their pH change during shelf life which is an indication of lactic acid production and number of increase in probiotic bacteria?"*

It was hypothesized that there will be a significant mean difference between the homemade and manufactured yoghurts in terms of lactic acid production during shelf life which is an indication of increase in number of probiotic bacteria. Homemade yoghurt will be the most acidic yoghurt, followed by the four different manufactured yoghurts.

To answer the research question and to test the validity of the hypothesis, pH values of the yoghurts which are incubated at 4ºC for seven days are measured by pH meter. Due to the increase in the number of probiotic bacteria, pH values of the yoghurts change because those bacteria make fermentation which produces lactic acid. The data is analyzed in order to specify if there is a significant difference after seven days of incubation in terms of the acidic property of the yoghurts.

As a result, yoghurts are placed in petri dishes and their pH values are measured at the end of 7 days (for control the data is taken each after 24 hours). Anova results showed that there is a significant mean difference between the homemade and the selected manufactured yoghurts which were incubated at 4ºC with respect to their pH change after 7 days incubation.

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1-Introduction:

It is certain that yoghurt is a kind of food which is not only very important for human health but also has high nutrition values. The minerals in yoghurt are very necessary and vital for us. For example, calcium and phosphorus are very significant minerals in yoghurt.[1]-[2] In addition to these, it has an effect to help digestion. Although some people have difficulty in digesting milk, they do not face with this problem after eating yogurt as some part of lactose is hydrolized during its fermentation[3]-[4].

Probiotics are living organisms that are beneficial for human health. It is found mainly in dietary supplements and foods. Lactic acid bacteria are also probiotic. Especially the lactic acid bacteria called *Lactobacillus bulgaricus* and *Streptoccocus thermophilus* take place in the formation of yoghurt. These bacteria are very useful for our body. "Bulgaricus in the dairy industry is identified as a "starter culture" that encourages the growth of other probiotic microbes during the production of yoghurt and cheese."[5] In other words, *Lactobacillus bulgaricus* prevents the production of harmful bacteria in the yoghurt and used as a "starter culture". Because of lactic acid produced by the bacteria, yoghurt becomes acidic and it does not let any microbes to appear[6].

The *L.bulgaricus* and *S.thermophilus* bacteria have important roles in human body. "*Streptococcus thermophilus* also lacks genes which contain surface proteins. This is important because harmful bacteria use these surface proteins to attach to mucosal tissues and hide from the body's defensive actions."[7] Moreover, " *L.bulgaricus* is one of the symbiotic microorganisms that can shrink or multiply within the environment of the mucous lining in the gastro-intestinal tract, also called intestinal mucosa. This environment is described in medical journals as an interference between the absorbtion of needed nutrients and the diversion of harmful microbes and toxins". [8] All these explanations may help us to understand the importance of *S.thermophillus* and *L.bulgaricus* for our body.

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[1] http://en.wikipedia.org/wiki/Calcium_metabolism
[2] http://e.wikipedia.org/wiki/phosphorus
[3] http://www.tgdf.org.tr/turkce/index.php?option=com_content&view=article&id=52&Itemid=68
[4] Probiotics protection against infection, Casey Adams, Phd
[5] http://www.probiotic.org/lactobacillus-bulgarıcus.htm
[6] http://www.probiotic.org/lactobacillus-bulgarıcus.htm
[7] http://www.probiotic.org/streptococcus-thermophillus.htm

[8] http://www.probiotic.org/lactobacillus-bulgarıcus.htm

Yoghurt is well known and available in Turkey. Personally, I am interested in yoghurts because I have heard several news about it from commercials and articles. In addition, my mum makes yoghurt at home rather than buying it from supermarkets because she thinks that homemade yoghurt is healthier for children. However, why does she think like that? Why do commercials and articles include lots of news about yoghurt? These questions have made me interested in this subject and I have decided to focus on manufactured and homemade yoghurt. I decided to compare manufactured yoghurt and homemade yoghurt in terms of lactic acid production which is an indication of presence of probiotic bacteria.

The packed yoghurt has a several processes during its production stage. These processes take place in hygienic places as they aim to prevent yoghurt to have unhealthy and harmful organisms. Because of this reason packed yoghurt is pasteurized. "Pasteurization is a process of heating of a food usually liquid to a specific temperature for definite length of a time and then cool it immediately .This process slows microbial growth in food".[9] In other words, during the pasteurization process, the harmful bacteria in yoghurt are damaged because of the high heat. On the other hand, not only the harmful bacteria but also the beneficial ones are damaged.[10] [11] Due to this reason, the packed yoghurt gets soured late. In other words, not being too acidic and not having enough lactic acid bacteria, the shelf life of manufactured yoghurt is longer.

The homemade yogurt does not have a pasteurization process during its production. As a result of this, the lactic acid bacteria are not damaged and can be found in the yoghurt. The yogurt becomes acidic and gets soured. This is an indication of lactic acid production due to the increase in the number ofand presence of lactic acid bacteria. This shows that there are more probiotic bacteria in homemade yoghurt when it is compared with manufactured yoghurts. The *L.bulgaricus* and *S.thermophillus* bacteria play an important role in yoghurt making and they are very important and useful bacteria for human body.

[9] http://www.en.wikipedia.org/wiki/pasteurization
[10] http://www.probiotic.org/streptoccocus-thermophillus.htm
[11] http://aboutyoghurt.com/Live-culture

According to all these information that are given above my research question is *" Is there a significant mean difference between homemade and manufactured yoghurts in terms of their pH change during shelf-life which is an indication of lactic acid production due to the presence of probiotic bacteria ?*

2-Hypothesis:

Yogurt is a healthy food which has been made for years. It has very important minerals for human health. It also includes the lactic acid bacteria called *Lactobacillus bulgaricus* and *Streptoccocus thermophilus*. These bacteria are known as very useful for human health. The people who even have lactose intolerance can digest yoghurt as some part of lactose is hydrolized during its production. This makes a lot of people prefer yoghurt to milk and they consume yoghurt more easily[12].

Yoghurt is not a food which is only produced in factories. It can easily be made at home as well. There are several differences between the homemade yoghurt and the yoghurt made in factories. The main reason of these differences is the way how it is made. The packed ones are made in hygienic conditions and they are pasteurized. This phase is applied to kill the harmful microorganisms in yoghurt. On the other hand, the probiotic bacteria can be damaged during this process as well.

In addition to this, the very important bacteria called *Lactobacillus bulgaricus* and *Streptoccocus thermophilus* can also be damaged, as it was indicated previously, although they contribute a lot for the production of yoghurt. When these bacteria are damaged, lactic acid production decreases and it becomes harder to destroy the microbes. These bacteria are very useful for human body. We cannot get benefit from them when they are damaged during the pasteurization process.[13]

On the other hand, probiotic bacteria (*L.bulgaricus* and *S.thermophillus*) in homemade yoghurt are less damaged because of not being pasteurized. The lactic acid bacteria reproduce more in homemade ones and due to this lactic acid production increases. In this respect, even if microbe comes into yoghurt, it disappears with the help of lactic acid. The other benefit is that, more probiotic bacteria are got into the body by the consumption of homemade yoghurt and this gives a lot of benefit to our body.

In the light of this information, it was hypothesized that there will be a significant mean difference between the homemade and manufactured yoghurts in terms of lactic acid

[12] Probiotics protection against infection, Casey Adams, PhD
[13] http://aboutyoghurt.com/Live-culture

production during shelf life which is an indication of increase in number of probiotic bacteria. Homemade yoghurt will have more lactic acid or in other words it will become more acidic at the end of seven days if it is compared with manufactured yoghurts. So it can be said that homemade yoghurt is healthier than the manufactured yoghurts in terms of having more probiotic bacteria.

3-METHOD DEVELOPMENT AND PLANNING:

The aim of this experiment is to determine the differences between homemade and manufactured yoghurt in terms of their lactic acid production during shelf life which is an indication of increase in number of lactic acid bacteria by measuring change in pH values. In this experiment, the acidic levels are taken into account in order to compare and contrast lactic acid production. Acidic levels are directly proportional to lactic acid production. That's why, when the acidic property of the yoghurt increases, the pH value of the yoghurt decreases. Based on the acidic property of the yoghurt, lactic acid production and the increase in the number of probiotic bacteria can be specified. To measure the pH values of the yoghurt, a pH meter is used.

Both manufactured and homemade yoghurt samples are placed into petri dishes and their pH values are measured by a pH meter. At the same time each day (every twenty four hours), pH values are measured again in order to learn the differences between the pH values and determine the acidic property of each yoghurt. This process is completed at the end of seven days. Last pH measurements which were done at the end of seventh day were taken into account to compare the pH change of different types of yoghurts and data analysis was done according to those measurements.

Thus, the independent variable is the type of yoghurt (manufactured and homemade). The dependent variable is the pH value. In other words, for each type of yoghurt, the lactic acid production during a period of seven days of shelf life should vary. The variability of pH value is based on acidity. That's why the variability of pH value can be related with the lactic acid production that is directly proportional with the presence of lactic acid bacteria, as it was indicated previously.

Comparing the pH values of each yoghurt type allows for the determination of the acidic properties of each yoghurt type. In other words, according to the presence of the lactic acid bacteria, pH values of each yoghurt changes. Their pH values can be measured by using a pH meter. In this experiment, the relation between different types of yoghurt and their acidic property based on lactic acidic production in a limited time frame during shelf life will be analysed.

In the supermarkets the yoghurts are kept in specific fridges and stored there until their expiration date. Keeping those yoghurt samples in a stable environment in terms of temperature was one of the key points of my experiment. I have measured the temperature of refrigerator at home and evaluated that the temperature fluctuation was so high (4 to 10ºC). So I decided to use more specified equipment and searched for it. For this purpose I used a cooled incubator (Heraus, Germany) and set the temperature of the incubator to 4ºC in Biological Science Department at Middle East Technical University.

For all groups and trials, the temperature and the amount of yoghurt are constant. The petri dishes containing the yoghurt samples are incubated reflecting normal yoghurt storage conditions and all samples' weights are measured by using the identical scale. Each yoghurt sample is stored under the identical conditions in order to avoid unwanted variables.

HOMEMADE YOGHURT RECIPE USED:

1- Procure carton of milk (1 liter)
2- Pour milk into a pot and bring to a boil.
3- Once the milk comes to a boil, remove the pot from the and set aside to cool.
4- Add a table spoon of natural yoghurt (obtained from village near Ankara, Kazan) to the pot and the yoghurt is mixed in very carefully with the cooled milk and also the mixture should be totally homogeneous.
5- The pot is covered with a cloth and let stand for a day so that the yoghurt can ferment.

MATERIALS:

1- pH meter (±0,005)
2- Petri dish (×20)
3- weighing scale (± 0,005gr)
4- natural yoghurt
5- Four different brands of manufactured yoghurt (Sütaş, Pınar AOÇ, Danone)
6- a cooled incubator (Heraus, Germany)
7- distilled water

METHOD:

1- Petri dishes are numbered from one to twenty.
2- 50 grams of homemade yoghurt is placed into the first five (1-5) Petri dishes.

3- Four brands of manufactured yoghurt are selected, Sütaş, AOÇ, Pınar and Danone.
4- 50 grams of AOÇ yoghurt is placed into the Petri dishes numbered 6,7,8,9,10.
5- 50 grams of Sütaş yoghurt is placed into the Petri dishes numbered,11,12,13,14,15.
6- 50 grams of Pınar yoghurt is placed into the Petri dishes numbered 16,17,18,19,20.
7- 50 grams of Danone yoghurt is placed into the Petri dishes numbered,21,22,23,24,25.
8- The pH values of all the yoghurt samples are measured using a pH meter. After every measurement, the pH meter is carefully washed in distilled water in order to restore the pH meter to neutral settings.
9- All pH values are noted.
10- When measurements are finished, all of the yoghurt samples are placed into cool incubator set at 4ºC.
11- This process is repeated for seven days and the data is recorded every twenty four hours from the initial testing was done.
12- The last pH measurements taken at the seventh day are used for calculating the pH changes for each typed of yoghurt and these measurements are taken into account for data analysis.

Picture 1: pH meter measures the pH values of the yoghurt

Picture 2: Petri dishes containing different brands of yoghurt

4- RESULTS:

Group	Trials	Initial pH (±0,005)	Final pH(±0,005)	Temperature(ºC)±0,5	Weight of yoghurt(gr)±0,005	Time period of shelf life(day)
Homemade yoghurt	1	4,500	4,170	4,0	50,000	7,0
	2	4,560	4,210			
	3	4,570	4,200			
	4	4,540	4,210			
	5	4,520	4,160			
AOÇ	1	4,700	4,470	4,0	50,000	7,0
	2	4,690	4,500			
	3	4,640	4,450			
	4	4,730	4,480			
	5	4,660	4,460			
Sütaş	1	4,800	4,540	4,0	50,000	7,0
	2	4,850	4,530			
	3	4,830	4,470			
	4	4,860	4,580			
	5	4,810	4,560			
Pınar	1	4,870	4,360	4,0	50,000	7,0
	2	4,860	4,400			
	3	4,840	4,430			
	4	4,800	4,370			
	5	4,820	4,460			
Danone	1	4,650	4,400	4,0	50,000	7,0
	2	4,600	4,330			
	3	4,480	4,170			
	4	4,620	4,320			
	5	4,660	4,380			

Table1: This table reflects the initial and final pH values of the yoghurt samples. First group is homemade yoghurt, second, third and the fourth groups are manufactured yoghurt, which include AOÇ, Sütaş, Pınar and Danone respectively. Uncertainties are determined by using the half of the least unit of the pH meter.

5-DATA ANALYSIS:

HOW CAN CHANGE IN pH BE CALCULATED?

First of all, in order to compare the pH values of the yoghurts, change in pH during a period of 7 days should be known. That's why, the values of change in pH is calculated. To illustrate;

The first trial of the homemade yoghurts has an initial pH which is 4,500 and has a final pH which is 4,170. When 4,170 is subtracted from the initial pH 4,500 and the result gives the value of change in pH which is, for this example, 0,33.

Group	Trials	Initial pH (±0,005)	Final pH(±0,005)	Change in pH	Average pH change
	1	4,500	4,170	0,33	
	2	4,560	4,210	0,35	
	3	4,570	4,200	0,37	
	4	4,540	4,210	0,33	
homemade	5	4,520	4,160	0,36	0,348
	1	4,700	4,470	0,23	
	2	4,690	4,500	0,19	
	3	4,640	4,450	0,19	
	4	4,730	4,480	0,25	
AOÇ	5	4,660	4,460	0,20	0,212
	1	4,800	4,540	0,26	
	2	4,850	4,530	0,32	
	3	4,830	4,470	0,35	
	4	4,860	4,580	0,28	
Sütaş	5	4,810	4,560	0,25	0,292
	1	4,870	4,360	0,34	
	2	4,860	4,400	0,34	
	3	4,840	4,430	0,35	
	4	4,800	4,370	0,31	
Pınar	5	4,820	4,460	0,30	0,328
	1	4,650	4,400	0,25	
	2	4,600	4,330	0,27	
	3	4,480	4,170	0,31	
	4	4,620	4,320	0,30	
Danone	5	4,660	4,380	0,28	0,282

Table 2: The above table indicates the pH change among the different yoghurt types. Group one is the homemade yoghurt, the other groups are the manufactured yoghurts, which are AOÇ, Sütaş, Pınar and Danone respectively. As the table shows, that homemade yoghurt has the most pH change, meaning that it is the most acidic. Pınar is the most acidic among the manufactured yoghurts.

	Homemade Yoghurt	AOÇ	Sütaş	Pınar	Danone
mean	0,348	0,212	0,292	0,328	0,282
std dev	0,01914854	0,03	0,040311	0,017321	0,019149
t- value	2,77644511	2,776445	2,776445	2,776445	2,776445
std error	0,00957427	0,015	0,020156	0,00866	0,009574
CI % 95	0,05316488	0,083293	0,111922	0,048089	0,053165

Table 3: This table indicates Descriptive Statistical details for each experimental group. The data that is given above is obtained using Microsoft Office Excel 2010.

Matching of groups	p- value	Existence of significant difference (P<0,05)
Homemade yoghurt vs AOÇ	0,02223346	YES
Homemade yoghurt vs Sütaş	0,03170709	YES
Homemade yoghurt vs. Danone	0,00273115	YES
Homemade yoghurt vs Pınar	0,01887478	YES

Table5:T-test pair wise calculation of p-values in order to identify the significant difference.

H_0: There is no significant mean difference between the homemade and the selected manufactured yoghurts with respect to their pH change during a period of 7 days at 4ºC.

H_a: There is a significant mean difference between the homemade and the selected manufactured yoghurts with respect to their pH change during a period of 7 days at 4ºC.

Anova: Single Factor

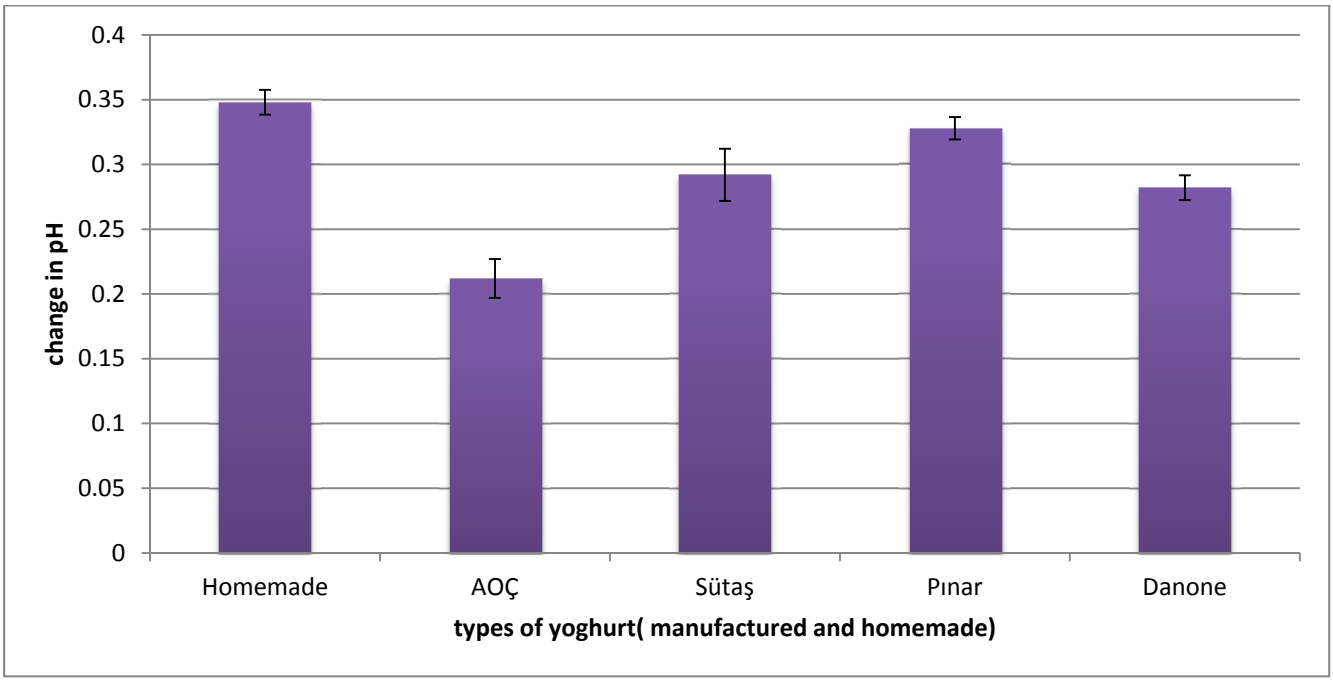
summary

Groups	*Count*	*Total*	*Mean*	*Variance*
Homemade yoghurt	5	1,74	0,348	0,00032
AOÇ	5	1,06	0,212	0,00072
Sütaş	5	1,46	0,292	0,00177
Pınar	5	1,64	0,328	0,00047
Danone	5	1,41	0,282	0,00057

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
between groups	0,054656	4	0,013664	17,74545	2,32E-06	2,866081
within groups	0,0154	20	0,00077			
total	0,070056	24				

Table 4: ANOVA results help us to determine whether H_0 or H_a is applicable. To determine which is the correct hypothesis, the p-value is analysed, which is 2,32E-4. It is seen that 2, 32E-6 is less than 0.05, therefore H_0 is rejected and H_a is the correct hypothesis for this experiment.



Graph 1: The graph shows the pH values of different types of yoghurt, homemade and manufactured yoghurt after seven days incubation at 4ºC.

6-EVALUATION:

The aim of this report was to investigate the difference among an assortment of yoghurts based on their lactic acid production, in other words, by examining their acidity levels. Measurements are analysed using the pH values in order to determine the change in pH to show varying levels of lactic acid production formed by lactic acid bacteria. It was hypothesized that there would be a significant mean difference of lactic acid production between the homemade and manufactured yoghurts during a 7-day period of shelf life.

Homemade yoghurt showed the highest acidity level. The manufactured yoghurts (Pınar, Sütaş, AOÇ and Danone) showed acidic property in a decreasing order, respectively. The pH value of homemade yoghurt varied between 0,33-0,37, with a mean value of 0,35. Meanwhile, the pH value of the first type of manufactured yoghurt, Pınar, varied between 0,31 and 0,35, with a mean value of 0,34. The second manufactured yoghurt, Sütaş, varied between 0,26 and 0,35, with a mean value of 0,30. The third manufactured yoghurt, AOÇ, had a pH value that varied between 0,19 and 0,25 with a mean value of 0,21.Finally, the fourth manufactured yoghurt Danone, varied between 0,25 and 0.31.

The ANOVA analysis helps to determine whether the null hypothesis (H_o) or the alternative hypothesis (H_a) is applicable to the results of the trail. This decision is made by using the p-value that is calculated using ANOVA. The null hypothesis is that there is no significant mean difference between the homemade and manufactured yoghurt with respect to their pH change. As the p-value is 2,32 E-6 that it is smaller than 0.05, the null hypothesis is rejected and alternative hypothesis is applied. That is; there is a significant mean difference between homemade and manufactured yoghurt with respect to their pH level change.

My hypothesis, which is there will be a significant mean difference between homemade and manufactured yoghurt in terms of lactic acid production during a 7-day period of shelf life, is supported by the results of the experiment and data analysis.

Some of the results were not expected during the course of the experiment. For example, I didn’t expect that the first manufactured yoghurt, Pınar, would have a pH value as close as it was to homemade yoghurt. This shows that Pınar has also a high level of

acidity, so there is a significant amount of lactic acid bacteria present. This also reveals that the lactic acid production for Pınar is higher compared to the selected manufactured yoghurts tested.

After analysing all groups, considerable differences between the pH values were recorded. In other words, the production of lactic acid was different for each yoghurt type grouping, reflecting that the varied presence of lactic acid bacteria in each group.

When I was making the experiment there were no unexpected circumstances that may affect the results of the experiment. However, I realized some possible errors in the method that may affect the results. To illustrate;

1- I measured the pH values of the yoghurts so that according to the pH values, I predict the amount of the presence of lactic acid bacteria (such as, homemade yoghurt is more acidic than the other types of yoghurts so that the number of lactic acid bacteria is much than the other types because it produces more lactic acid that cause yoghurt to become acidic.) However, if the number of the lactic acid bacteria were counted by using agar plate zone then I obtained more distinct results and this results may help me to decide which yoghurt is more healthy.

2- Temperature is very significant factor that affects bacteria, directly. Each species of bacteria has unique optimum growth temperature so if the temperature is not suitable for the bacteria, it will die and won't display activity. For lactic acid bacteria optimum growth temperature changes between 34ºC and 36ºC (the reason of this change is, there are two types of lactic acid bacteria that present in the yoghurt so their optimum temperature is different from each other) Because of this reason if the experiment is done in the room temperature(25ºC), lactic acid bacteria reproduce more so the lactic acid production is higher when it is compared with my results. That means if the experiment is made in the room temperature, the efficiency of the bacteria increases so pH change is observed clearly. In addition, if some microbes reproduce in the yoghurts, the lactic acid bacteria kills them because they produce lactic acid and because of the presence of lactic acid in the yoghurt microbes cannot grow.

7-CONCLUSION:

My research question, “Is there a significant mean difference between homemade and the selected manufactured yoghurts in terms of their pH level during shelf life based on the lactic acid production recorded at the same time every day for seven days, and at a constant temperature of 4ºC?”, is answered by the results of the study. It is found that there is a significant mean difference between homemade and manufactured yoghurt in terms of their pH levels, so it can be said that lactic acid production which, is an indication of presence of lactic acid bacteria, is variable. As predicted, homemade yoghurt becomes more acidic than the manufactured yoghurts tested.

The reason why I have chosen this subject is because I wanted to understand better the overwhelming amount of information regarding yoghurt presented in news articles, commercials and advertisements. I have read some articles discussing the differences between homemade and manufactured yoghurt, arguing which yoghurt is healthier. This debate interested me, so I decided to try and answer the question myself. By using a pH meter, I measured pH values of the homemade yoghurt and compared them with manufactured yoghurts. The reason why I compared the pH values of the homemade and the selected manufactured yoghurts was to determine which type of yoghurt becomes more acidic which is an indication of increase in the number of probiotic bacteria. By measuring the pH values, I could also comment on the amount of lactic acid bacteria present in the varying yoghurts. As previously mentioned, probiotic bacteria contains health benefits for the human body so it can be said that homemade yoghurt is healthier than manufactured yoghurts in terms of having more probiotic bacteria since it becomes more acidic during shelf life. In addition, during the process of preparing the homemade yoghurt, I didn’t use any food additives such as gelatin, which is considered harmful to the human body. However, gelatin is used in some of the manufactured yoghurts, which makes the yoghurt consistence and not so healthy.

All taken into account, yoghurt is a traditional food that has several advantages for human wellbeing. In the light of this research, it is seen that, from the pH values of the yoghurts, homemade yoghurt becomes more acidic than the other manufactured yoghurts. This shows us that the number of probiotic bacteria in homemade yoghurt is more than the

others. It was mentioned previously, probiotic bacteria, *L.Bulgaricus* and *S.Thermophillus*, are beneficial for human. In addition, homemade yoghurt does not include food additives and food preservatives so it is natural. For all these reasons, it can be said that homemade yoghurt is healthier than manufactured yoghurts.

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