TED ANKARA COLLEGE FOUNDATION HIGH SCHOOL

Investigating the effect of fat concentration on the acidity of spoiled milk

Biology Extended Essay

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Abstract

As we all know economy shapes our life; it is everywhere including education, living standards, social life, health sector etc. In many countries economy doesn't affect health service people get. However, in Turkey, it is a big issue. Food, drinks and even healthcare services people get depend on their socioeconomic status. In most urban parts of Turkey people drink unpasteurized milk. They don't know the spoilage date of milk and sometimes physical characteristics alone (acidity, loss of emulsion property etc.) can't be enough to understand that milk has spoiled. Therefore, people may consume spoiled milk and get sick. Especially it is more difficult to detect spoilage signs in low fat milk. Usage of milk is very common in Turkey. Many of us use milk in their cooking, it's a fundamental nutrient for all of us and it is really important in our diet too. In TV commercials we see lots of warnings about dangers of consumption of spoiled milk because in urban parts of Turkey especially, each year lots of people get poisoned because of this. In this essay, I investigated the research question "How does fat concentration affect pH of milk after spoilage?" To be able to answer this question, I used three different milk types of the same brand with different fat concentrations and measured their acidity after spoilage. It was hypothesized that there will be a significant pH difference between the pH of the milks with a different fat concentration because of the dissolving rate of carbon dioxide in fat. Milk with greater fat concentration will be the most acidic after spoil. The hypothesis was supported by experimental evidence. Although the initial pH values were the same, the average final pH values were 6.22 for full fat milk, 6.38 for medium fat milk and 6.52 for low fat milk indicating that the greatest pH change was observed in full fat milk.

I-Introduction

Many dairy products such as milk contain probiotics which consist of bacteria that are helpful to our gastrointestinal system. They keep the balance of the intestine and they have digestive benefits. The most common type of bacteria which helps our gastrointestinal tract is called lactic acid bacteria such as Lactobacillus acidophilus. In our daily life we consume those types of bacteria in yoghurt, milk, kefir and they do not have any known serious harm. However, these products also contain harmful bacteria called coliforms. Coliforms refer to a group of bacteria including *E.coli* Most of these bacteria are destroyed when milk is pasteurized but some of them stay alive and they start to spoil the milk. After its expiration date we can see significant changes caused by those bacteria. Typical physical characteristics of milk which have spoiled are; sour taste, loss of emulsion characteristic (liquid under the solid top), increase in the acidity and bad smell.

Milk consumption in Turkey is very important and there are still people who are consuming unpasteurized milk in the urban parts of Turkey. With my essay I want to show that coliforms are very important in consumption of milk and they spoil milk even if it is pasteurized and if milk is unpasteurized they are more dangerous and sometimes we do not realize whether the milk is spoiled or not by looking at its physical characteristics only. Spoiled milk is dangerous because it harms our gastrointestinal system and millions of dollars are spent in hospitals in a year for curing it. "United States Centers for Disease Control (CDC) estimates that food poisoning costs the United States between 5 and 6 billion dollars annually in direct medical care and lost productivity. In undeveloped countries it causes 4-6 million deaths each year." In spoiled milk harmful bacteria starts an infection which causes "diarrhea, fever, abdominal pain, and nausea and muscle pain." So my research question is “How does fat concentration affect pH of milk after spoilage?”

The above information led me think that it is hard to identify the characteristic of spoiled milk which have low fat concentration because there won't be a significant change in the taste of the milk based on its acidity. Increase in acidity is caused by the carbon-dioxide which is excreted by the coliforms. Coliforms in milk consume minerals and other nutrients in milk and as a result they excrete carbon-dioxide as a metabolic waste. Carbon-dioxide decreases the pH of the milk but we see that pH's are different in milks which have different concentrations of lipids.

Carbon-dioxide is a symmetrical molecule which has non-polar property and because of its molecular shape and polarity it dissolves in lipid efficiently because lipids have also non-polar polarity. As a result of this chemical property of those substances, milk with different lipid concentrations have different pH values when they spoil. Full fat milk will be more acidic after it spoils because of the rate of dissolving of carbon-dioxide in fat and extra-light milk will be the least acidic one and vice versa. If this hypothesis is proved, people should be more careful about consuming low fat milks without looking its expiration date.

II. Hypothesis

My research question is “How does fat concentration affect pH of milk after spoilage?” In milk two bacteria are always present; Lactobacillus acidophilus and Escherichia coli. Lactic acid is the natural bacteria in milk which doesn’t make any harm to human body but the dangerous ones are coliforms including Escherichia coli which are the reason why milk spoils. We can kill those bacteria with pasteurization but some of them still survive and if these bacteria gain a suitable environment which promotes their growth, they will rapidly multiply which causes milk to spoil. (1)

When the milk starts to spoil, there is a formation of carbon-dioxide because of the metabolic activity of the Escherichia coli (respiration). Formation of carbon-dioxide as a metabolic waste decreases the pH of the milk. As a non-polar molecule it dissolves in non-polar molecules rapidly and efficiently and since the lipid molecules are non-polar carbon-dioxide dissolves in them and as a result it increases the acidity.

In the light of this information, it is hypothesized that there will be a significant pH difference between the pH of the milks with different fat concentrations because of the dissolving rate of carbon dioxide in fat. Therefore, as fat concentration decreases, carbon-dioxide dissolves less and, pH change is less. It is predicted that the acidity of the milk which has the highest fat concentration will be the highest value. So my hypothesis is that milk with greater fat concentration will be the most acidic after spoil.

III. Method Development

My research question is “How does fat concentration affect pH of milk after spoilage?” and it is hypothesized that milk with greater fat concentration will be the most acidic after spoil. To test my research question and decide whether my hypothesis is correct or false, data must be obtained and compared with statistical tests and different mathematical formulas.

Selection of milk type that will be used was the first issue that I had to concern. To keep variables like initial pH and sugar concentration constant, I decided to use same milk brand. After looking lots of milk brands, my search revealed that Pınar brand had milk with different fat concentrations all of which have the same protein content, sugar content and initial pH value. This brand had milk with three different fat concentrations; milk with full fat concentration (3.4 gr/lt), light milk (1.5 gr/lt) and extra light milk (0.1 gr/lt). The reason why I decided to use commercially available forms of milk with different fat concentrations is that it is really hard to change the fat value of only one milk because addition of fat to milk is a complex procedure and in order to be efficient lots of constant should be known about fats such as constant percentage values of addition of protected fat, seed fat, natural fat etc.

The next step was letting milk spoil in same environment and in the same conditions. Same volume of each type of milk should be put it in sterile beakers to prevent any contamination to make sure that it is the coliform bacteria that remain in milk after pasteurization that causes spoilage, rather than bacterial contamination from the environment. It is hard to keep the entire values constant because spoilage of milk is affected by many variables; the temperature, sugar levels present in the milk, carbon-dioxide and oxygen concentration in the environment. Those conditions affect the spoilage quickly because they directly affect the Escherichia coli’s rate of reproduction and there is a direct correlation

between the reproduction rate of the coliforms and the carbon dioxide level present in the milk. To keep the temperature constant, I decided to use an incubator which also helps to keep oxygen and carbon-dioxide levels constant in the environment. To keep the sugar values constant milks with same percentage of sugar should be chosen. Using an incubator was the easiest and cheapest technique to keep temperature, oxygen and carbon-dioxide levels constant because it is a machine which is used frequently in all laboratories especially in micro-biology and cell biology labs.

8 days is the optimum time for this kind of milk to spoil [(1)] so experiment should be finished at the end of the 8th day.8 days later physical changes are observable because milks have spoiled. When milk spoils it gives a sour smell, it forms a clot because of the acidity and there is a slight change in the color of the milk [(2)] (it seemed like a little bit lucid, it has lost its emulsion property). I have adjusted incubators temperature to 25^{o}C because it is the optimum time for milk spoilage [(1)].

Using pH indicator strips for calculating the pH is subjective and inaccurate therefore for more precise calculations use pH meter (Digital pH Meter (Portable Type) HANNA HI-8424).

1- http://wiki.answers.com/Q/How_long_will_it_take_for_milk_to_spoil_in_the_refrigerator_after_it_has_been_opened
http://orgs.usd.edu/esci/age/content/natural_clocks/milk.html
2- http://www.ehow.com/how-does_4572637_what-makes-milk-spoil.html

Materials:

Test Substances:

- Pınar milk with full fat concentration (3.4 gr fat, 1lt)
- Pınar milk light (1.5 gr fat, 1lt)
- Pınar milk extra light (0.1 gr fat, 1lt)

Materials to be used in the experiment:

- 250 ml sterile beakers x 15
- Volumetric flask
- Incubator (it keeps oxygen, carbon-dioxide and temperature levels constant in the container.)
- Plastic wrap (plastic cling film) x 15 (covers the top of the beakers, to block the interaction of milk with exterior environment.)
- Rubber bands x 15
- Marking pen
- Thermometer

Instruments:

- Digital pH Meter (Portable Type) HANNA HI-8424

IV. Method

Wear gloves during the experiment to prevent the contamination of the test system by external factors.

A. Preparation of the test substances

1. Get 15, 250 ml sterile beaker.
2. Label them one by one according to their fat concentrations.
3. Pour 200 ml of low, medium and high fat milk respectively.
4. Cover their surfaces with plastic wraps and fix it with rubber bands.
5. Put them into an incubator to keep their temperature constant. (Also it keeps oxygen and carbon-dioxide levels constant)
6. Keep beakers in 25^{o}C for 8 days in incubator. Temperature was first measured with Thermo Sensor Oregon Scientific and in the laboratory it was measured with normal thermometer.

B. Conducting the experiment

1. Pour milks in the beaker into the volumetric flask to check again whether their volume is exactly 200ml.
2. After pouring into the volumetric flask, pour milks into the sterile beaker with a thermometer in it.
3. Measure the temperature of the milks.
4. Take digital pH Meter (Portable Type) HANNA HI-8424 and dip into the milks.
5. In each dip measure the pH value and note down into a notebook.
6. Also in each dip clean the rod of the Digital pH Meter (Portable Type) HANNA HI-8424 with pure water to prevent data error in the experiment.

7. After dipping it to all 15 cups measure the temperature of the room.
8. Throw plastic gloves into the bin which has a paper which says “Experimental Garbage Only”. Clean the laboratory carefully because the experiment was with spoiled milk which can be dangerous to the human physiology.

C. Data Analysis

1. Calculate the means of the pH values of the milks.
2. Use Microsoft Office Excel 2010 to count sum, average and variance of the values.
3. Do ANOVA (Analysis of Variance) test to see if there is a significant difference between the mean of final pH values.
4. Use Independent T-test to see the mean differences of pairs.
5. Calculate the descriptive statistics which are SD (Standard Deviation), SE (Standard Error) and confidence interval.

VI. Data Analysis

The following formulas were used to calculate the mean, SD (Standard Deviation), SE (Standard Error) and CI (Confidence Interval) for the statistics of the experiment. (3)

1. Mean:

Mean =sum of data values/ number of data values

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

2. Median:

The median is the exact middle value of a set of data values that have been sorted from the lowest value to highest. If the number of data values even, then the median is the average of the two middle values.

3. Mode:

The mode is the data value that occurs at a greater frequency than the others.

4. Range:

The range is the highest data value minus the lowest data values.

5. Variance:

Variance is used to measure how far the data is away from the mean. The distance of the data point from the mean is a deviation.

$$\sigma^2 = \frac{\sum_{i=1}^{n} (x_i - \mu)^2}{n - 1}$$

6. Standard Deviation

The standard deviation is just the square root of the variance.

$$\sigma = \sqrt{\frac{\sum_{i=1}^{n} (x_i - \mu)^2}{n - 1}}$$

7. Confidence Interval

95%CI = SE x t (n-1)

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

V. Results

Fat Concentration (gr/lt)	Trial Numbers	Initial pH	Volume of Milk (ml)	Temperature (^{O}C)	Final Ph	Protein (gr/lt)	Energy(kcal/kj)
3.4	1	6.6	200.0	25.0	6.2	3.10	61.8 / 259
	2	6.6	200.0	25.0	6.2	3.10	61.8 / 259
	3	6.6	200.0	25.0	6.3	3.10	61.8 / 259
	4	6.6	200.0	25.0	6.2	3.10	61.8 / 259
	5	6.6	200.0	25.0	6.2	3.10	61.8 / 259
1.5	1	6.6	200.0	25.0	6.4	3.20	45.9/192
	2	6.6	200.0	25.0	6.4	3.20	45.9/192
	3	6.6	200.0	25.0	6.4	3.20	45.9/192
	4	6.6	200.0	25.0	6.3	3.20	45.9/192
	5	6.6	200.0	25.0	6.4	3.20	45.9/192
0.1	1	6.6	200.0	25.0	6.5	3.30	34.1/142
	2	6.6	200.0	25.0	6.6	3.30	34.1/142
	3	6.6	200.0	25.0	6.5	3.30	34.1/142
	4	6.6	200.0	25.0	6.5	3.30	34.1/142
	5	6.6	200.0	25.0	6.5	3.30	34.1/142

Table 1: Initial pH, volume of milk, temperature, protein content, energy value and final pH for each trial of each fat concentration.

FAT CONCENTRATION	MEAN	MODE	MEDIAN	RANGE	VARIANCE	SD	SE
Full Fat Milk (3.4 gr/lt)	6,22	6,2	6,2	0,1	0,002	0,04472136	0,020
Light Milk (1.5gr/lt)	6,38	6,4	6,4	0,1	0,002	0,04472100	0,019
Extra Light Milk (0.1gr/lt)	6,52	6,5	6,5	0,1	0,002	0,04472136	0,020

Table 4: Descriptive statistics including mean, mode median, range, variance, SD (Standard Deviation), SE (Standard Error)

Anova: Single Factor

SUMMMARY

Groups	Count	Sum	Average	Variance
Column 1	5	31.1	6.22	0.002
Column 2	5	31.9	6.38	0.002
Column 3	5	32.6	6.52	0.002

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

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.225333	2	0.112667	56.33333	7.95E-07	3.885294
Within Groups	0.024	12	0.002			

Total	0.249333	14				

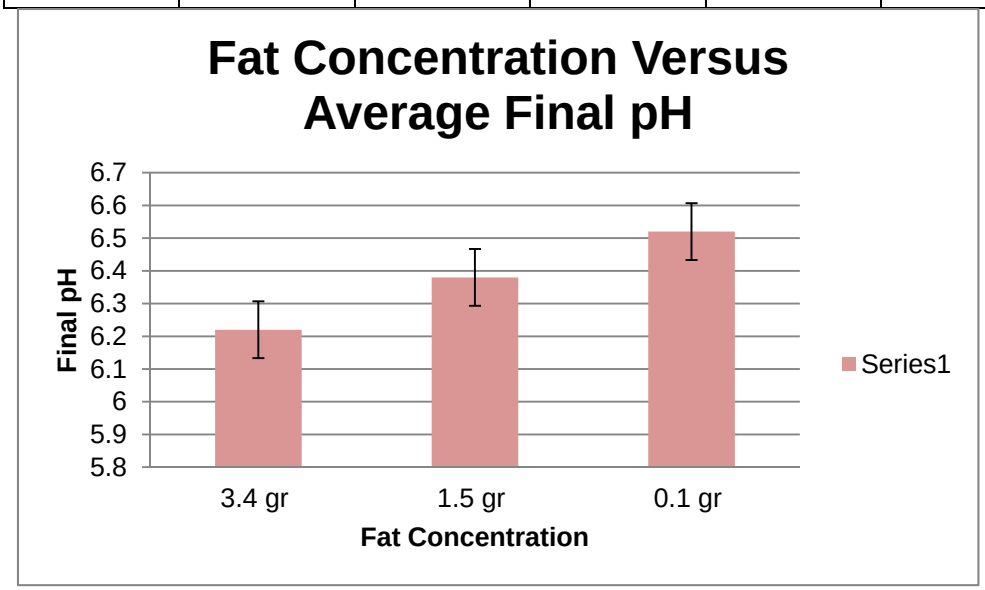

Table 5: The comparison of pH values with milks with different fat concentrations

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

Matching Groups	P-value	Existence of significant difference (P<0.05)
Full Fat Milk-Light Milk (3.4 gr/lt)	6,83495195921107E-07	Yes
Full Fat Milk-Extra Light Milk (1.5gr/lt)	5,36115973862945E-08	Yes
Light Milk-Extra Light (0.1gr/lt)	1,05786777664674E-06	Yes

Table 6: Independent t-test (two sample assuming equal variances) results used to compare the existence of significant difference between two groups.

VII. Evaluation

The research question of this investigation was “How does fat concentration affect pH of milk after spoilage?” So the aim of this study was to find out whether there is a significant difference in acidity between milks with higher concentration of fat and lower concentration of fat. Depending on their fat concentration it was hypothesized that there will be a difference in acidity of the milks and milk which has the highest fat concentration will be the most acidic one. Milks with high fat concentration (3.4 gr/lt), medium fat concentration (1.5gr/lt) and low fat concentration (0.1 gr/lt) has been used and according to my hypothesis the low fat milk (0.1 gr/lt) is expected to have the less pH change. Our experiment had showed us that the predicted results and our hypothesis are correct because 8 days later milk with high fat concentration had a 0.38 pH change, medium fat concentration had a 0.22 pH change and low fat concentration had a 0.08 pH change. (All had same initial pH 6.6)

These results showed that all the milk which has spoiled has an increase in their acidity and the milk with highest concentration of fat has the highest value of acidity. In order to find whether there is a significant difference or slight difference of acidity between three milk groups (full fat milk, light milk and extra light milk) ANOVA and t test (two samples assuming equal variances) had been used. After making the t test, pH showed that there is a significant difference between full fat milk, light milk and extra light milk. (Even though we don’t see a huge difference between the values those differences are important for acidity values.) When I compared the mean of the groups, I saw that extra light milk had the highest mean which shows us that there wasn’t a huge change between the initial acidity level and the final acidity level. By this information we can understand that the spoiled milks with lowest fat concentration is not too acidic so we can’t understand by looking at the taste of an extra

light milk if it is spoiled or not. The means of the full fat milk, light milk and extra light milk were 6.22, 6.38, and 6.52 which shows that spoiled full fat milk is the most acidic.

My null hypothesis was that spoiled milk with the highest fat concentration level won't be the most acidic one. Three test groups and the control group were tested by ANOVA and p-value calculated from it is 7, 95- E which was smaller than 0.05 so my null hypothesis was rejected and my hypothesis "Spoiled milks with greater fat concentration will be the most acidic" was accepted. Fat concentration changes the pH because of its molecular structure. As we all know "like dissolves in like" efficiently and faster therefore carbon-dioxide as a non-polar molecule dissolves in fat (non-polar molecule) efficiently. So, there is a direct correlation between the fat concentration of milk, carbon dioxide dissolving rate and pH change

The results of SD and SE had results as I have expected. The SD (Standard Deviation) values are small according to means of the test group which shows us that the acidity values were distributed homogenously. Standard error values are a little bit small than SD (Standard Deviation) values. This shows that there were some errors in the experiment. However, there wasn't any problem in the conduction of the experiment yet there can be some systematic errors caused by the method. There were not random errors caused by the experimenter.

The problems that may have occurred in the experiment can be listed as follows:

1. Protein (gr) difference between the products: Since spoiled milk has many bacteria (*coliforms*), they can use proteins for their benefit or for food which can cause difference in reproduction and fatality rate of the bacteria in spoiled milk with different protein and fat concentration.

2. Calorie (kcal/kj): Since milks that had been chosen have different fat concentration they won't give the same calories. Also, in all dairy products there is a direct correlation between calorie values and fat concentration.

Limitations of this extended essay:

1. The brands of the milk were Pınar, so the type of the preservatives was same. However, I didn't make the experiment with other milk brands so my extended essay is limited with only one brand.
2. The bacteria which spoils the milk and changes the acidity of the milk is *Coliform* but there are many types of those bacteria so it is a limitation to my extended essay because I can't find all kinds of the *Coliform* in the spoiled milk so I had to generalize it.
3. I do not know the chemical properties of the preservative which has been used in the milk so I cannot predict whether it affected spoiled milks acidity or not.
4. The experiment was made with milks with different fat concentrations. The main reason why the acidity increases in milk is that carbon dioxide dissolves in fat faster so the milk with the most fatty acid concentration will be the most acidic. However, fatty acid concentration can also affect the reproduction rate of the bacteria which I cannot prevent.

VIII. Conclusion

My research question “Do the milk which has the greatest fat concentration will be the most acidic after spoil?” was answered with the results of the experiment and statistical analysis of the results which showed us that acidity of the spoiled milk has a direct correlation with the fat concentration. The hypothesis which was made was correct and according to the statical analysis we can say that the experiment was a success although standard error was a little bit high. As I have explained its reason in the VIIth part, protein (gr) and calorie (kcal/kj) values were different in high, medium and low fat concentrated milks. The conditions which have contributed to this result should be investigated more deeply (All kinds and effect of the *Coliforms* etc.) so that exact reason of acidity can be explained.

The reason why I chose to deal with this subject is that people always think pasteurized milk is perfectly healthy. However, after the pasteurization some of the *Coliforms* stay alive which is the main reason why milks spoil so all people should be very careful about their way of preserving milk and consuming it before its spoil time. In Turkey we see lots of commercials about the importance of milk or how to preserve milk by the Health Ministry. They make those commercials because many people are still using the unpasteurized milk and they don’t know its danger. They think that after buying the unpasteurized milk if you boil it, you will kill all the bacteria. However, it is not true if you boil the milk you can’t kill the harmful bacteria efficiently instead you kill the beneficial components and denaturalize the protein which makes milk more of a nutrient with negative effects, I wanted to show the importance of the milk in our life by making the extended essay. The results showed me that all people should consume milk carefully and they should know consumption differs from fat concentration because of the spoilage rate. It is important because many people consume diet milk or extra diet milk for their fitness but they do not know how to preserve it correctly.

Reference

3- http://wiki.answers.com/Q/How_long_will_it_take_for_milk_to_spoil_in_the_refrigerator_after_it_has_been_opened

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