INTERNATIONAL BACCALAUREATE DIPLOMA PROGRAMME

BIOLOGY

EXTENDED ESSAY

EFFECT OF FAT CONCENTRAION IN PASTEURIZED MILK ON REPRODUCTION RATE OF ESCHERICHIA COLI

SCHOOL NAME: TED ANKARA COLLEGE

SUPERVISOR NAME: Gonca ESENDEMİR

CANDIDATE'S NAME: İrem KUŞÇU

CANDIDATE'S NUMBER: 001129-0046

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ABSTRACT

As pasteurized milk was accepted to be free from any type of bacteria contamination in the beginning, the different ingredients in different ratios can cause reproduction of several bacteria. According to nutrition amount, reproduction rate and number can differ from each other. In this experiment, fat concentration in milk was taken as independent variable as fat being a long-term energy source. Thus, the purpose of this study was to examine the effect of fat concentration on milk which leads my research question *How does different fat concentration of milk (100% and 0.1 %) affect the reproduction of Escherichia coli in Mueller Hinton agar plate by using the method dilution in 1/100 ratio and plating?* Although there are several methods for determining the number of bacteria, counting method was easiest to apply and quickest to obtain results. However before starting to experiment, it is important to know if milk contains any other substances to avoid systematic error. Moreover, before planting *E.coli,* the suspension should be diluted and than planted to a large area of agar plate for planting and counting method to be more accurate. In order to verify results of an experiment for statically, t-test should be done. In anlaysis processi t-test result was found $1.37x\ 10^{-15}$ which can be concluded that the hypothesis is proven to be correct and fat concentration in milk effect bacteria reproduction

1. INTRODUCTION

The mammals produce milk for the essential nutrition for their newborns. The white colored liquid is the fundamental food for them until the time they can digest the other type of nourishment. In worldwide, multiple dairy farms are on progress to pasteurize milk in order to decontaminate from toxic chemicals. For human consumption, different type of milk is produced like whole, fat-free, organic and lactose-free milk by synthetically. For bacteria milk used for energy and biosynthesis of macromolecules while these ingredients contribute the human body and its development due to its rich content.

Milk is slightly odor and opaque with the combination of water, fat particles, protein particles and lactose addition of minerals and vitamins. Cow milk has a pH value changing between 6.4 - 6.8, which is the reason why milk is slightly acidic. The most abundant compound in the structure of milk is water with the mean 87 %. Milk is rich with the type of minerals and the vitamins although they are only 8-10% existed. The commonly known calcium helps to strengthen bones and teeth with vitamin D. Vitamin B-12 produces red blood cells which are responsible of the carriage of oxygen from lungs to muscles. Minerals are generally used for balancing the pH value, milk's osmotic pressure and also the ionic strength. With the value of 4.8 % lactose is the source of carbohydrate in the milk. While their main duty is production of energy, when lactose are broken the products glucose and galactose can cause milk to ferment to lactic acid at suitable environments. With the fraction of 3.4 %, there are lots of different types of proteins in milk. Proteins are essential to fight diseases while producing new cells and contributing the production of muscles. The primary groups of milk proteins are caseins with phosphoproteins, lipoproteins and

chromoprotein. In the case of addition of acid or the presence of an acid-producing bacterium, the pH value will be decrease and precipitations occur. Consequently, milk is curdled.

Lipids form 3.9 % of milk's structure. The fatty acid in the structure are very distinctive, they have the ability to adapt their amount of fat in order environmental qualities. The main milk fat is triglycerides, which is the combination of one glycerol molecule and three different types of fatty acids. At 37° C, milk fat's state changes from solid to liquid. Due to cows' body temperature is also 37° C, the milk fat is in their liquid state at the body. Triacylglycerol form fat globules when they are together. Every lipid in the milk is secreted with the shape of fat globule and being covered with a membrane. In milk, one of the fats main contributions is flavoring. They can absorb flavor and can completely change the taste. Hydrolytic rancidity is one of the two types. In hydrolytic rancidity, with enzymes fatty acids are the products of broken down of glycerol molecules. The new occurred fatty acids affect the taste of the product. Other type, oxidative rancidity, is a result of oxidation of fatty acids. When fats are oxidized, milk's flavor become greasier and tallow. Since there is a brood spectrum of carbohydrate, protein and lipid types and amounts; it is logical to expect more than one type of milk, which are mainly divided according to abundance of particles in their structure as well as their production process. Though one single process called pasteurization is essential for each type of milk regardless to their fat rate.

Pasteurized milks are heated and clarify from their any harmful bacteria however the flavor remains same. This type is the most common one all around the

world due to easiness of usage. The essential part here is that, keeping them in cold environment and it must been consumed in two or three days. Unpasteurized milks also present. Although, some people choose to use this type, amount of unknown bacteria can cause serious diseases. On the other hand, whole milk can be thinking between these two types. They are freshly from cow however they are mostly pasteurized. In another approach, milks can be separated according to their fat concentration. While normal ones contain 3.9 %; semi-skimmed only contains 1.5-1.8 % and in skimmed milk there are nearly no fat. However, on the down side with the reduction of fat, also some vitamins that dissolve in fat like the vitamin A and D are also being removed from the milk. Knowing that fat is a long-term energy source, and can be used in case of starvation, fat concentration in milk can be identification for different types of bacteria growth.

Bacteria are in length of a few micrometers, a benefiting factor for being oldest living organism, and can be found with different shapes. Their habitat differs according to their structure and some of them are enable to adapt their selves. In every nutrition different types of bacteria present which are specialized to several of functions. While some of them are beneficial some of them causes severe diseases. Most of these bacteria exist on the raw milk and the products made of raw milk. Even pasteurization decreases the risk of contamination, still at different fat levels; milk gets spoilt which suggests reproduction of bacteria. Despite the fact that there are millions of bacteria around and maybe there are hundreds of bacteria at our daily milk. Incontrovertible positive effect on our life, their destruction for human should also be considered. Some of them may not be cause a severe harms however their consequences weaken the human immune system, which may lead other disease.

Bacterial enzymes such as *Pseudomonas, S.lactis, Mycobacterium tuberculosis* are commonly found in milk and can result spoilage, acid fermentation which leads to fatal diseases. *Escherichia coli* also presents in milk but pasteurization can prevent to present and reproduce. Still they can be present which causes diseases such as Hemolytic Uremic Syndrome, Guillain-Barre Syndrome, gastroenteritis, urinary tract infections, and neonatal meningitis.

Escherichia coli is a prokaryotic organism that is found mostly intestine of human or animal in healthy conditions. They are gram-negative, which means they do not retain the crystal violet dye a procedure to distinguish bacteria), and facultative anaerobic (they produce ATP by aerobic respiration when oxygen is available in the environment, but if oxygen could not be found this bacteria can produce ATP by fermentation of anaerobic respiration as well) rod-shaped bacteria. While some of them can be beneficial by producing K_2 vitamins, some of them can be fatal due to food poisoning. The symptoms vary from mild and water to severe and bloody diarrhea, to abdominal cramping to vomiting.

In the light of this information, bacteria should be at suitable environment for growth and therefore it needs nutrients. As already mentioned before, lipid is the most energizing organic substance, which causes fat ratio to be a determinative factor. The aim of this experiment is to determine the relationship between fat ratio of pasteurised milk and reproduction of *Escherichia coli* bacteria. Therefore my research question is *How does different fat concentration of milk (100% and 0.1 %) affect the reproduction of Escherichia coli in Mueller Hinton agar plate by using the method dilution in 1/100 ratio and plating?* We can not be one hundred per cent sure that our

food supplies are free from bacterial influences. The risk is still intact despite heating process. On 7 December 2012, an article was published by Brandon L. Jutras, Alicia M. Chenail and Brian Stevenson. As they examined bacterial growth rate can be influenced by temperature, they developed a method allowing them to evaluate Borrelia Burgdoferi's growth rate according to protein amount in the environment. As their experiment results, nutrient rate can limit bacteria's development. Thus, my hypothesis suggests that bacteria suspension in small fat ratio would have smaller number of reproduced bacteria than bacteria suspension in whole milk. They will be able to find more nutrients for their growth until the time they divide by mitosis or conjugation and therefore they will reproduce more rapidly and continuously.

In order to perform a controlled and manageable investigation, the experiment will take place at a microbiology laboratory in *Atatürk Hastanesi* with contributions of *Tuba Müderris*. With the help of a specialist, laboratory environment and specialized materials; the experiment should have minor errors.

2. METHOD DEVELOPMENT

2.1. Preparation of the Experiment

While conducting this experiment, my aim was to determine the relationship between nutrient amount and cell growth. Therefore my research question is "*How does different fat concentration of milk (100% and 0.1 %) affect the reproduction of Escherichia coli in Mueller Hinton agar plate by using the method dilution in 1/100 ratio and plating?"* The bacteria suspension, which is planted to milk with 100% fat should have biggest ratio of reproduction of *E.coli* according to given information. *Escherichia coli* would have more nutrient to sustain its development therefore it should reproduce more in number in the limited time than milk with 0.1% fat ratio. The independent variable in this experiment is fat concentration in same brand of milk. The reason for choosing milk for this experiment is its variety of other forms like cheese, yogurt etc. we use in daily life. It is an easy material to find and work on it. Milk can get easily spoiled which means strongly affected by bacteria thus the results of this experiment would be prominent and represents possibility of a wild spread disease caused by the different amounts of lipids in milk and food poisoning. Moreover having a large range of fat concentration would be adjuvant. Number of reproduced *E. Coli* will be counted, as it's the dependent variable of the experiment.

Escherichia coli is a prokaryotic model organism for biotechnology and microbiology due to it is being a host organism in studies with recombinant DNA. As *E. coli* being responsible for food poisoning, we can conclude that it can react with milk as well in this experiment. At laboratory environment and for purpose of this experiment, *Escherichia coli* is the primary source. It is ability to grow easily and without causing too much time and money result the choosing of this bacteria. Also

human beings are very informed about it which makes it easy to investigate in addition to high possibility of serving as a disease agent.

Carbohydrate, protein and lipid are presented in milk and these products are all essential for every organism. While carbohydrate gives the least amount of energy lipid gives the most energy when it was broken down, and protein can differ according to organism. These reasons lead to choosing fat for the experiment. Furthermore, finding different fat concentrations milk is more accessible than carbohydrate and protein. As fat was taken independent variable, 100% and 0.1 % ratios can be taken for more prominent results.

There are several methods for counting bacteria. First one is counting chamber which is a kind of microscope slide which enables you to count the number of a cell culture. Secondly bacteria's electrical resistance feature is examined using a Coulter Counter. This method applied to count the number of cell and measure their volume. According to shown resistance to electricity which should be none accepting that they conduct the electricity. Spectrophotometry is a method determining the number if bacteria according to turbidity of bacteria suspension. The most common method and the one that I am going to use is counting bacteria by dilution and plating. This principle is based on adding extra fluid to bacteria suspension than plating this solution to a Petri dish which have nutrition to allow growth and reproduction of bacteria. After given time, initial bacteria number increase and would be appropriate to count them by eye or using microscope according to dilution rate and bacteria type. This way is easiest one and therefore easiest to get accurate feedback.

In order to obtain more accurate and precise outcomes of the experiment; there are several other controlled variables through the procedure. For instance same branded milk are used. Thus, while volume of fat for 100 mL milk changes other variables remain constant for milk. 200 mL milk box are bought but only 60 mL milk is required for the experiment. Anything related to milk apart from fat concentration is kept constant by using same branded milk. As initial number of bacteria would be important for results of the experiment due to limited habitat and fat concentration can cause competition, thus it should be identical. Number of bacteria was kept constant comparing bacteria suspensions' turbidity to Mc Farland 0.5 which has universally accepted bacteria number at a suspension with 1.5×10^8 numerical value. After preparing suspension of *E.coli* and milk, diluting it and planting to agar dash; these dashes should be kept at 36.1 Celsius in vacuum furnace as 36.1 Celsius is ideal for *E.coli* because it is average human body temperature and *E.colis*' main habitat is human or animal body. The temperature in vacuum furnace remains constant by electric. All Mueller Hinton agar dashes are kept at vacuum furnace for 24 hours, as this time period being adequate for bacteria to grow and reproduce, which allows us to stabilize the time for reproduction.

Bacteria suspensions are diluted 1/1000000 in overall; after adding it to two different fat concentration milk, they are diluted both 1/10 and 1/100 ratio again for all agar dashes. Dilution was essential for counting method to be definite and less time consuming. 1/100 dilution rate was chosen to investigate because they can spread the whole area of agar dishes while containing less number of bacteria than 1/1o dilution ratio. Rather than counting millions of bacteria from Mc Farland 0.5, this process enable us to obtain more evident numbers. Furthermore, only 1 cm^2 area

of agar plate was counted, than compared to the whole area. These two processes assist us to have definite results and they are less time consuming. Only one type of dilute was used in order to create equivalence. Physiological serum, a solution of sodium chloride in water, will be used for its sterilizing quality and because for each trial same amount of saline is going to be used there will be no difference in nutrient amount apart from planted milk type. In total 2 L of physiological saline was used for dilution and 200 mL for each trial in each fat ratio.

Identical materials are used each trial however one material does not used again in order to not transmit any other type of bacteria or transmit *E. Coli* from other suspensions. So that bacteria growth and reproduction will be caused by differences in fat concentration. Identical Hinton Mueller agar dashes are used so that each of them has equal nutrients apart from fat concentration coming from milk. Although there are several agar plates mainly derivatives of blood agar plates; in this experiment Mueller Hinton, contains beef infusion, peptone, and starch, will be used because of its bigger radius which simplify the counting process. Sterile urinals were used to reserve milk and bacteria suspension due to its lack of contamination from other organisms. For each trial, milk bottle was shaken and 60 mL milk was taken so that equivalence can be achieved apart from fat concentration.

Centrifuge will be used until obtaining homogeneous solution which should take no more than 1-2 minutes so that when bacteria numbers were counted and proportioned to the whole agar plate, results will be distinctive. 1 microliter of milk-bacteria suspension will be put in Mueller Hinton plate which leads to even though there are millions of bacteria, the counting would be easier because of the small

amount taken. In addition to homogeneous suspension, agar plate also has to be homogeneous. Thus, using round-ended loop 1 microliter will be spread to every part of plate. Each Hinton Mueller agar plate will be stay in vacuum furnace for 24 hours in 36.1 Celsius stabilized temperature as it is accepted as optimum inhibition temperature for *E.coli* because it is average human body temperature, which is habitat of *E. coli*, and optimum time frame for bacteria to grow and reproduce.

2.2. MATERIAL LIST

- A metal scissor
- Round-ended loop
- 2 L physiological saline
- 60 mL whole milk (x5 for each trial)
- 60 mL 0.1 % fat concentrated milk (x5 for each trial)
- Sterile urinal (x20 for each trial)
- Test tube (x20 for each trial)
- Mueller Hinton agar plate with 14 cm radius (x20 for each trial)
- Syringes
- Autoclave
- Vacuum furnace
- Centrifuge
- A metal 30 cm ruler ($\pm$ 0.1)

2.3 PROCEDURE

1. A metal scissor is sterilized by using the heat of autoclave
2. Tip of 200 mL milk boxes from same brand are cut by using sterilized scissor
3. For 0.1 and 100 % fat concentrated milk, an amount of milk is poured into two different petri dishes
4. Each of them are examined under a microscope in order to make sure the milk is 100% pasteurized and there is no bacteria living it which can cause errors in experiment
5. 200 mL of physiological saline is put in a test tube using a syringe
6. Round-ended loop is sterilized by using heat of autoclave
7. From previous experiments, *E. coli* bacteria is taken from an agar plate by using round-ended loop and the bacteria sample is put in two test tubes
8. Dilution and planting method has been applied (see appendix A and B)