Effect of temperature on bacterial growth in milk

Abstract

The purpose of this experiment was to understand the conditions which encouraged and inhibited bacterial growth by observing the rate of bacterial reproduction in milk samples stored at different temperatures. The results collected after the six day period of observation revealed that factors which influenced the rate of bacterial growth were temperature, pH level, moisture and the amount of food sources available. It was concluded that milk is an ideal setting for the reproduction of bacteria due to its high water content, pH value close to 7, and ingredients. Warmer temperatures provoked bacteria to reproduce quicker as opposed to cold temperatures which restricted bacterial growth. Furthermore, the lactobacilli bacteria existent in milk produced lactic acid by fermenting the sugars in milk. As time passed by, this lowered the pH level of the milk samples, which caused the milk to curdle and produce a pungent sour smell.

Introduction

The Kingdom Bacteria consisted of anaerobic unicellular microorganisms with exceptional abilities of adapting to wide ranges of environment conditions. Bacteria were the only living organisms that existed on Earth for approximately 3. 5 billion years implying that these prokaryotic organisms were able to survive through all the harsh climate changes in Earth’s history. Furthermore, bacteria existed everywhere; in the deserts and the oceans, in glaciers and hot springs, in the bodies of other living organisms and even in the Earth’s atmosphere (“ Bacteria,” 2014).

Classification of the Bacteria Kingdom was based on the shape, structure/thickness of cell walls, sources of food and energy, and the analysis of RNA sequences. Bacteria could obtain energy from various sources. They could be photoautotrophic, photoheterotrophic, chemoautotrophic, or chemo- heterotrophic. Additionally, these organisms came in several different shapes; the most common being cocci (round), bacilli (rod-shaped), and spirilli (spiral shaped) (“ Classification of bacteria,” 2012). Each shape offered distinctive advantages. Coccus had protection against drying out, bacillus bacteria had great surface area for nutrients absorption and spirilla bacteria were able to travel through fluids with ease. Moreover, bacteria commonly grew in distinguishing arrangements. Some grew in pair (diplo), some in clusters (staphylo), and others grew in a chain (strepto) (“ Bacterial shapes and,” 2014).

A population of bacteria could increase greatly in a fairly little amount of time considering that they reproduce exponentially. In favourable conditions, bacteria would reproduce asexually through a type of cell division process called binary fission. During this method of asexual reproduction, the single DNA molecule would replicate to create a copy of its original single chromosome. As the cell continued to grow, it would elongate and begin to form a septum between the two DNA molecules. Finally, a distinct cell wall would be formed between the two DNA molecules splitting the original call into two smaller genetically identical daughter cells (Bailey, 2014). In unfavourable conditions, a few bacteria were able to reproduce sexually by a process called conjugation with the intention of increasing the survival probability. This process allowed new gene combinations to be introduced

which may provide the daughter cells a better chance of adapting to the changing conditions. During conjugation, two bacterial cells with slightly different genetic make-ups were connected to each other through protein tube structures called pili. The donor bacterium would transfer all or part of its chromosome to the receiving bacterium. The receiving cell would then undergo binary fission with the new gene content to produce more cells with this new gene combination. There were also other types of reproduction that bacteria go through such as transformation, transduction, and spore formation (Galbraith et al., 2001).

Purpose

- To understand the favourable and unfavourable conditions for bacterial reproduction
- To monitor and compare the rate of bacterial growth in milk stored at warm and cool temperatures

Materials

- 8 glass cups
- pH paper
- Plastic Wrap
- Skim Milk
- Whole Milk
- Chocolate Milk
- Buttermilk

Procedure

1. 4 glass cups were labeled with the different types of milk: whole, skim, butter, and chocolate.
2. The other 4 glass cups were labeled as; whole, skim, butter, and chocolate as well.
3. A small sample of the different types of milk (~50mL) was poured in the corresponding labeled glass cups.
4. 8 observation tables were created; 2 tables for each type of milk; fridge vs. cupboard.
5. The temperature was taken for each sample of milk and recorded in the appropriate observation table.
6. The pH level was taken for each milk sample and recorded in the appropriate observation table.
7. The odour of the samples of milk was described and recorded in the correct observation table.
8. The colour of the milk samples were identified and noted in the corresponding observation table.
9. A description of the appearance of the milk samples were made and noted in the appropriate observation table.
10. 4 glass cups each with a different type of milk sample were placed in the fridge.
11. The other 4 glass cups filled with different types of milk samples were placed in a cupboard/cabinet.
12. The steps 5-11 were repeated for 6 days
13. At the end of the experiment; on the 6 th day, all samples were disposed of properly and all glassware was thoroughly cleaned.

Discussion

The results after the six day observation period showed that the four types of milk which are kept in the fridge underwent little to no changes in appearance, odour, pH level or colour, whereas the milk that were left in the cupboards presented drastic changes in all aspects. The chocolate milk in both the fridge and the cupboard seemed to have undergone the greatest amount of change after the six days compared to the other three types of milk left in the corresponding conditions. Among the four types of milk left in the fridge, the chocolate milk and the butter milk were the only samples that went through a slight colour and appearance change. Among the samples left in the cupboard, the skim milk and chocolate milk showed the greatest amount of difference in appearance from day 0; the beginning, to day 6; the end of the experiment. All samples of milk dropped in pH levels and it seemed like by the fourth day, all samples kept in the cupboards had produced a cheese-like solid substance that was surrounded by a thin liquid residue.

Milk has ideal conditions for bacterial growth having high water content, plentiful nutrients, and a pH level that’s very close to neutral (6. 4-6. 8). Also, the substances in milk such as sugar (lactose, glucose), milk fat, protein, and other compounds provided the bacteria with a great amount of food supply for energy. The bacteria present in milk could be arranged into two main groups; pathogenic, and spoilage bacteria. Pathogenic bacteria induced food poisoning such asBacillus cereus whereas spoilage bacteria were only capable of producing pungent odours, unappetizing flavours, and changes in texture and appearance of milk. The most common bacteria found in milk

were the lactobacilli. These bacteria converted the sugar in milk (lactose) into lactic acid (“ Biochemical changes in,” 2014).

There were several factors that affect the rate of bacterial growth. The most significant factor proven in this experiment was temperature. Several food agencies reported that at temperatures between 5 to 60°C several foodborne bacteria were able to grow; this was referred as the “ danger zone.” This fact explained the reason behind the extremely slow rate of bacterial reproduction in the fridge milk samples considering that refrigerators were usually kept under 4°C to inhibit rapid bacterial growth in the foods. Furthermore, research showed that food bacteria reproduce the quickest at temperatures ranging between 21 and 47°C. Hence, the bacteria in the milk samples kept in the cupboards were multiplying at a much faster rate than the milk samples in the fridge. For that reason, after about two days, the subject would be able to notice distinctive changes in the odour and appearance of the milk samples from the cupboards (“ Danger zone,” 2014).

The nutrients in the four different types of milk were also a big factor that influenced the rate of bacterial growth. Chocolate milk had about 10g of sugar per 100mL (“ Chocolate milk,” 2014), whole milk had about 5. 2 grams (“ Nutrition facts,” 2014), skim milk had 4. 9g (“ Skim milk,” 2014), and buttermilk with a 5 g sugar content per 100mL (“ Buttermilk,” 2014). Based on the sugar content of the milks, it was shown that the rate of bacterial reproduction in the chocolate milk was faster than the other types of milk because of its high sugar content which was about double the sugar content of the other types of milk. The lactobacillus bacteria existent in milk

fermented the carbohydrates such as glucose and lactose in milk into lactic acid. Therefore, the greater amount of available food supply, the faster the bacteria multiply in the milk (“ What is lactobacillus?,” 2014).

The pH values were another factor that affected the bacterial reproduction in the milk samples. Most bacteria preferred to live in a pH value of 7; neutral. The pH levels of all the milk samples excluding the buttermilk, were very close the neutral, therefore they encouraged bacterial growth. However, due to the low temperature that the samples in the fridge were kept in, the bacterial growth was restricted, hence less lactic acid was produced maintaining the pH levels of the milks close to 7, while the samples kept in the cupboards all dropped in pH levels significantly; became more acidic after the six days. This was because both the warm temperature and the close to neutral pH level provided the bacteria in milk, lactobacilli, with a favourable environment for reproduction allowing more bacteria to ferment lactose which produced more lactic acid and increased the acidity of the milk (“ Microbiology,” 2014).

However, most bacteria cannot survive in very acidic environments; a low pH level. As the increasing bacteria population in the cupboard milk samples continued to produce lactic acid, they were creating an unfavourable condition for themselves. Therefore, after a while the cupboard samples would slowly increase in pH level; become more basic, as all the bacteria die due the extremely acidic environment and thus the bacterial growth rate would decrease drastically. At this point, the milk would be more vulnerable spoilage caused by mould and yeast which are able to survive in pH values below 4. 5, extremely acid conditions (“ What pH is,” 2012).

The milk samples kept in the cupboards for six days produced a pungent sour odour while the samples in the fridge had no or a faint sour smell. This was because of the difference in the amounts of lactic acid present in the two groups of samples. A physical property of acids was sourness, therefore considering that there were more lactobacilli bacteria in the cupboard samples breaking down sugar and producing lactic acid as a by-product, there was a greater quantity of acid in the samples forming the strong sour smell. Whereas, the milk samples in the fridge had less amount of lactic acid present and produced a faint or no sour odour (Chua, 2008).

All the milk samples kept in the cupboard developed curd after the six day observations; this was because of the ingredients in milk. Milk contained various compounds, the main ones being fat, protein, and sugar. Milk was an emulsified colloid where the protein molecules are suspended and dispersed within a water-based solution. The colour of milk was due to the ability of these protein molecules to refract light. These protein molecules repelled each other, however when the pH level decreases, the molecules suddenly became attracted to each other forming chunks. This was what happened at the molecular level when milk developed curd. Hence, the clumps of a cheese-like substance that was formed at the end of the experiment in the cupboard samples were protein (casein) molecules. The greenish yellow liquid residue surrounding the floating clump was a solution of translucent whey. The curdling process of milk occurred more quickly at warmer temperatures compared to cold temperature, therefore only the samples in the cupboard developed curd (Moncel, 2014).

Conclusion

It could be concluded that several factors influenced the bacterial growth rate in milk. A few factors were temperature, pH levels, water content, and available food sources. The main one was temperature. Warm temperature provided bacteria a favourable environment for reproduction which caused them to multiply quickly as opposed to cooler temperatures. The pH values also affected the bacterial growth rate. Various bacteria could grow in an environment with the pH level close to 7; hence in a close to neutral environment, bacteria reproduce rapidly. However in milk, as the lactobacilli population grew rapidly, the amount of lactic acid increased greatly also, considering that these bacteria broke down the lactose in milk and released a by-product of lactic acid. This lowered the pH level greatly which caused the death of bacteria. Therefore, after a few days the pH values of buttermilk sample in the cupboards increased as more bacteria died and stopped producing lactic acid. The chocolate milk with the most amount of sugar content, which provided the bacteria more food supply, spoiled the quickest compared to the other types of milk. The rate of the curdling of milk is caused by both the warm temperature and acidic conditions in the cupboard milk samples. Lastly, the sharp sour odour of the cupboard samples after a few days were produced by the lactic acid present in the milk.

Errors

Errors which occurred during this experiment included inaccurate measurements of the pH values considering that the colours of the red litmus paper strip were difficult to differentiate. Also, different individuals might interpret the colours differently and for the buttermilk and whole milk, the values of the exact pH levels weren’t recorded. These errors affected the

analysis greatly since the information wasn’t specific and accurate. Furthermore, the results could’ve been different depending on how each individual perceived the changes that had occurred to the milk samples. Another factor that could’ve influenced the outcome was the different expiry dates for the types of milk. These errors would’ve altered the observations that were made significantly.

Applications

The factors that control the rate of bacterial growth discovered in this experiment could be applied to everyday life. For example, the production of sour cream, yogurt, and cheese were all results of the fermentation of milk where the lactobacilli broke down lactose in milk into lactic acid. The drop of the pH values in the milk caused by the lactic acid resulted in various different alterations of the milk, appearance and texture wise producing different fermented dairy products (“ Milk,” 2014).

Furthermore, foods should be kept refrigerated in order to inhibit bacterial growth. Foods which were kept in warmer temperatures such as in the oven for at least a day or two should not be consumed; they might be hazardous to one’s health considering that warm temperatures encourage bacterial reproduction. If the factors were considered, one would be able to control the growth of bacteria. Kitchen utensils and equipment should be kept clean and dry in order to restrict the amount of bacteria produced since moisture level, and the amount of available nutrients are both factors that affect the rate of bacterial reproduction (“ Dairy bacteriology,” 2013).