Microbiology Quality in Cheeses

Research Methods

Helen Arceneaux

2/22/14

Abstract

Cheese styles were compared to determine if certain styles were more likely to have *Escherichia coli* cultures. This experiment was conducted in two parts. In part one streak plates were created to compare the microbial potential in several cheese styles. For each style chosen there were two cheeses compared. The cheese styles tested were blue cheese, emmenthaler, manchego and gruyere. In part two, 3M petrifilm plates were used to determine if *E. coli* cultures existed on the cheeses selected. Most of the cheeses were found to have low potential for *E. coli* cultures. However, blue cheese was found to have the highest potential due to the high moisture content of these cheeses. This higher potential could also be attributed to the ripening process of the cheese. The younger the blue cheese the greater its potential to grow *E. coli*.

Introduction

All cheese comes from milk. There are endless ways of creating cheese which produces an enormous variety. Each variety demands the certain processes that define it (McSweeny 2004). For instance, soft-ripened cheeses rely on molds, yeasts and bacteria to ripen it from the outside in. Harder cheeses ripen from the inside out. There is also a difference in starter cultures, the aging process, milk sources and even the time of year created (Beresford 2001).

Recently cheeses have taken criticism. Food scientists advocate pasteurizing milk before the cheesemaking process and food lovers discuss how pasteurization limits flavors. Some critics argue that cheese should be avoided altogether for the fear of contamination during the aging process. To restrict possible food-borne illnesses in cheese, the USFDA restricts the sale of cheese that are made from raw milk and aged less than 60 days. There is some evidence that some pathogens are naturally occurring in cheese but at a low level or are part of a false positive test because of a large amount of DNA (Fluitt 1993).

Cheeses rely on bacteria to form. There are many kinds of bacteria used in starter cultures for cheese and some bacteria is introduced by the environment as it ages (Franklin 1963). Since cheeses provide a beneficial environment for certain bacteria to form, harmful bacteria growth must also be addressed. This experiment was created to test if the potential for microbial cultures of *Escherichia coli* vary with each classification of cheese. As cheeses age there is less moisture but more exposure to their environment.

Experimental Design

Streak plates were designed to catalogue the amount of bacteria growth on each cheese. The comparative tests were needed to determine the potential for *E. coli* cultures. Because the potential for bacteria growth was so large the 3M petrifilms were then used to identify whether *E. coli* existed on any of the cheeses. Both the agar plates and the petrifilms were incubated at 37C for 48 hours to ensure an equal culture time. Pairs of cheeses were tested and compared based on one variable.

Two cheeses, El Trigal and Artequeso, were chosen in the Manchego basket style of cheese supplied from Mitica. Each of these cheeses have the same maker, the same sources of milk, the same age time and the same supplier. They are formed in a basket with sheep's milk. Each is aged for 6 months. The testable variable for this set of cheeses is whether raw milk cheese, the Artequeso, has a higher or lower potential for pathogen

count than the El Trigal which is made from pasteurized milk. Both have the same milk source but one is pasteurized and one is not.

Emmi Roth supplier was chosen for the Gruyere style of cheeses. The exact same supply chain ensures the same shipping time and same source. This style of cheese is made with pasteurized cow's milk. The cave aged Gruyere and non-caved Gruyere were both tested for comparison from this maker. These two cheeses were chosen to see if the cave aging process increases the potential for E. coli cultures.

For the blue cheese comparison the Igor Mountain (Piccante) and the Igor Dolce varieties were chosen. These two cheeses have the same maker, supplier and milk sources. These cheeses are made from cow's milk. These two vary in aging style and this is the independent variable being tested. The Igor Mountain (Piccante) is cave aged. This additional cave age test was included because blue cheeses ripen differently than most cheeses and have large amounts of active bacteria. They require cultures of penicillin mold and other cheeses do not. Also, blue cheese ripens from the outside in versus the cheeses being tested which ripen from the inside out.

A pair of emmenthaler cheeses was also chosen. A pre-packaged Emmenthaler aged 180 days was compared to an Emmenthaler that was aged 3 months. The tested variable was age. Emmenthaler is a swiss style of cheese. The variable tested in this pair was if the age of the cheese changes the potential for bacteria cultures.

Materials

The cheeses tested all came from the same retailer.

- Inoculating loops qty 8
- Sterile swabs qty 8
- Agar plates qty 9
- Liquid agar
- Incubator set to 37C
- Small Beakers/containers qty 9
- 1 wrapped piece of each of the following cheeses:
  El Trigal Manchego 6 month aged pasteurized milk
  Artequeso Manchego 6 month aged unpasteurized milk
  Emmi Roth Gruyere 180 days aged, pre-packaged
  Emmi Roth Gruyere 6 months aged
  Igor Gorgonzola Dolce
  Igor Gorgonzola Mountain cave aged
  Emmenthaler
  Isopropyl alcohol

Procedure Overview

The test consisted of nine total agar plates. One agar plate is used as a control and will remain sterile. 3M *E. coli* indicator plates, for a total of nine, will be made in total. One indicator plate remains sterile as a control. Each cheese is exposed and swabbed with a fresh sterile swab. The swab is streaked across a sterile agar plate. An agar plate is made from each swab for a total of three plates for each piece of cheese. A cell count is performed on each plate after 3 days in an incubator at 37°C. Each cheese will be

numbered the following to correspond to the correct number of agar and 3M plate for comparison:

1	Manchego- 6 months raw milk
2	Manchego- 6 months pasteurized milk
3	Emmenthaler- aged 180 days
4	Emmenthaler- aged 3 months
5	Gruyere cave aged
6	Gruyere non cave aged
7	Blue Cheese- Mountain (Piccante)
8	Blue Cheese- Dolce
9	Control Plate

Part I: Agar plate preparation

Before the testing agar plates were made. Premixed agar was used in this test. After each plate cooled for 30-45 minutes the completed petri plates were numbered in sequential order from 1 to 9.

Each cheese was numbered on its wrapper in sequential order from 1 to 9 and the number each 3M petrifilm in sequential order from 1 to 9. Each cheese was tested in the corresponding number plates. (Please see experimental design for specific sequence used in this experiment.)

A slit was cut in the cheese wrapper labeled 1. The cheese was not fully unwrapped as to limit its exposure to the outside environment. The sterile swab was unwrapped at the cotton end. The cotton end of the swab was inserted into the slit in the cheese packaging. The cotton swab was rubbed many times over the surface of the cheese. The agar plate was gently streaked with the cotton swab. The cotton swab was disposed of in biohazard waste. The instructions were repeated for all eight cheese samples. One agar plate was left untouched as a control.

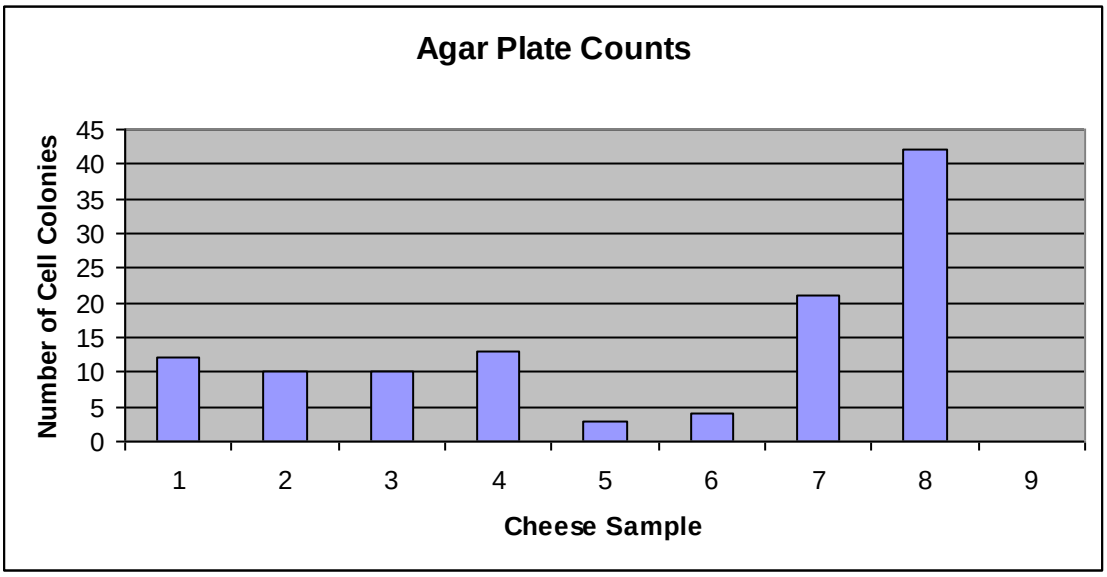
The agar plates were stored upside down to reduce moisture buildup. The plates were placed in the incubator for 48 hours. The agar plates were then removed and the plates were compared for bacterial growth.

Part II: 3M Petrifilm procedure

Each beaker and petrifilm was labeled in sequential order from 1-9. Next 10 ml of distilled water was distributed into each beaker/container using a 10 ml pipette. The inoculating loop was inserted into the slit on the cheese packaging previously made in part I for cheese labeled 1. The sample taken was then mixed into the beaker labeled 1. A small pipette was used to pipette out 1 ml of the mixture. The sample mixture was distributed under the film and slowly released to reduce any chance of air bubbles. The 3M spreader was used on top of the petri plate to spread liquid. The steps were then repeated for plates 2-8. Plate 9 was used as a control as was inoculated with distilled water only. The plates was placed in an incubator for 48 hours at 37°C. Isopropyl alcohol wipes was used to sanitize work area after placing the films in the incubator.

Data

The chart represents the cell count totals for part I of the experiment. No cell counts were collected in part II because all tests came back negative for *E. coli*.



Analysis of Results

There were nine petri plates in the first part of the experiment. Eight were swabbed and the ninth was used a control. This part of the experiment showed microbial growth of cheeses. Blue cheeses are still maturing on a microbial level when consumed and this microbial activity showed on the plates 7 and 8. Although the plates 1-6 all showed some microbial activity, it was very little due to low moisture and their ripening process.

Plates 1 and 2 had very similar bacterial growth. Plate 1 had twelve colonies and plate 2 had ten colonies. Plates 3 and 4 had ten and twelve bacteria colonies respectively. Plate 5 and 6 were also similar with 3 and four colonies. However, plate 7 and plate 8 had a large discrepancy in plate counts. Plate 7 was a sample of the aged blue cheese and plate 8 was a sample of the young blue cheese. Plate 7 had twenty-two colonies whereas plate 8 had forty three colonies.

In part I of the experiment, one of the petri plates was poured too thin. As a result, the agar was nicked when swabbed. This might have influenced the bacterial growth on that plate. In part II it was found that the 3M petrifilms do not work well if there are bits of solid in the liquid. The liquid is not spread as evenly. Also, the pipettes can clog with bits of solid which leads to less of a liquid sample or more of a liquid sample than wanted on the film. Additionally, only the round spreader should be used when spreading the film. Unknowingly a spreader meant for mold samples was used on the first few plates that caused some uneven streaking of the petrifilm. This streaking could have affected results. Images of the tests cane be found in the appendix.

If this experiment were done again the agar should be poured to a height of 4mm in each petri plate to ensure even streaking. Also, there should be a better procedure in place to liquefy the samples of cheese for use under the 3M pertifilm. A third part of the experiment could have been included to introduce *E. coli* to the cheese samples in order to verify the potential for pathogen growth on each cheese.

Conclusions

No *E. coli* was detected so broad generalizations can only be made on the bacteria growth found on the agar plates. Most of the cheeses were visibly low in moisture content. They were semi-hard or hard cheeses. Moisture is a key element needed for microbacterial growth, including *E. coli.* The results show that the higher moisture cheeses, the blue cheeses, have a higher microbial content. There may be other factors contributing to the bacterial growth. No conclusive evidence was found. While they did not test positive for *E. coli* these cheeses might be more favorable to *E. coli* growth due to the moisture content. Although the *E. coli* growth was negative, any positive growth could also have been attributed to food safety.

The original hypothesis was to attain the potential for *E. coli* comparatively in each style. A third part of the experiment that inoculated blue cheese with *E. coli* may have provided more insight.

References

Beresford, Tom, Nora Fitzsimons, Noelle Brennan, and Tim Cogan. "Recent Advances in Cheese Microbiology." *International Dairy Journal 11* (2001): 259-74. Web. 8 Feb. 2014. <http://comenius.susqu.edu/biol/312/recentadvancesincheesemicrobiology.pdf>.

Fluit, AC, R Torensma, MJC Visser, CJM Aarsman, and MJJG Popilier. "Detection of Listeria monocytogenes in Cheese with the Magnetic Immuno-Polymerase Chain Reaction Assay." *Applied and Environmental Microbiology 59.5* (1993): 1289-93. Web. 8 Feb. 2014. <http://aem.asm.org/content/59/5/1289.full.pdf>.

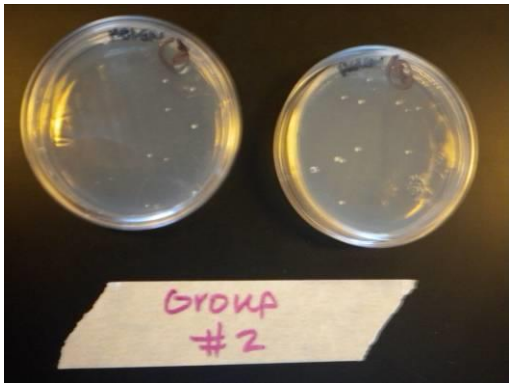
Franklin, J G., and M E. Sharpe. "The incidence of bacteria in cheese milk and Cheddar cheese and their association with flavour." *Journal of Dairy Research* 30.1 (1963): 87-99. Web. 6 Mar. 2014. <http://journals.cambridge.org/action/displayIssue?jid=DAR&volumeId=30&seriesId=0&issueId=01>.

McSweeney, Paul. "Biochemistry of Cheese Riping." *International Journal of Dairy Technology 57.2/3* (2004): 127-44. Web. 8 Feb. 2014. <http://comenius.susqu.edu/biol/312/biochemistryofcheeseripening.pdf>.

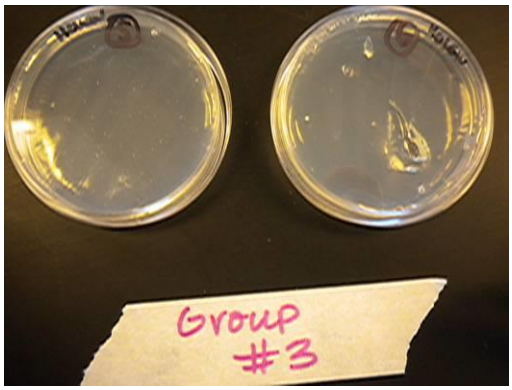
Appendix

Phase I: Agar Bacteria Growth Comparison Tests

Group 1: Manchego style cheeses comparison test
1. Manchego 6 month raw milk
2. Manchego 6 month pasteurized milk



Group 2: Gruyere style cheeses comparison test
3. Emmi Roth Gruyere Cave Aged
4. Emmi Roth Gruyere



Group 3: Emmenthaler style comparison test
5. Emmenthaler 180 days aged
6. Emmenthaler 6 months aged

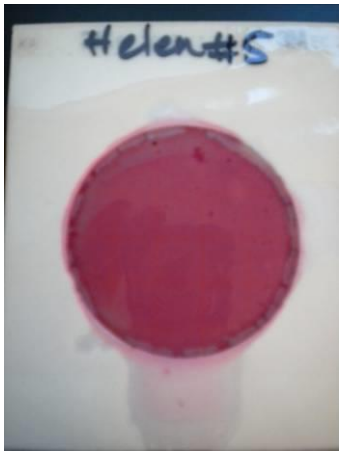
Group 4: Blue Cheese comparison test
7. Igor Gorgonzola Mountain (Piccante)
8. Igor Gorgonzola Dolce

Control

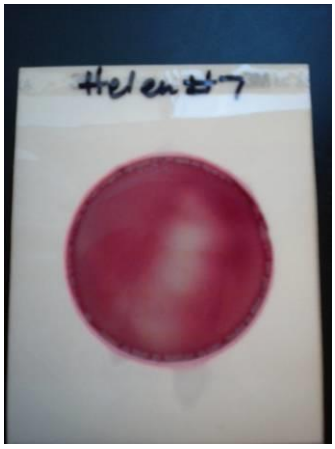
II: 3M Petrifilm E. Coli Comparison Test

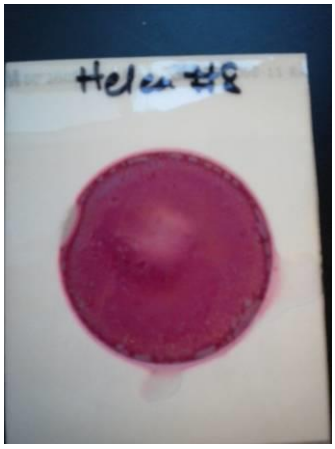
Group 1: Manchego style cheeses comparison test
1. Manchego 6 month raw milk
2. Manchego 6 month pasteurized milk

Group 2: Gruyere style cheeses comparison test
3. Emmi Roth Gruyere Cave Aged
4. Emmi Roth Gruyere



Group 3: Emmenthaler style comparison test
5. Emmenthaler 180 days aged
6. Emmenthaler 6 months aged





Group 4: Blue Cheese comparison test
7. Igor Gorgonzola Mountain (Piccante)

8. Igor Gorgonzola Dolce

9. Control Plate