IB Biology

Cow Milk Spoilage in Five Different Temperatures

Purpose:
Investigation of the rate of cow pasteurized milk as measured by the pH spoilage in different temperatures of 1˚C, 5˚C, 7˚C, outside temperature (average 10 ˚C) and room temperature (20˚C). And determining the best temperature for storing milk.

Research Question:
How does the temperature in which milk is stored affect its pH?

Hypothesis:
The milk stored in the temperature of 1˚C is expected to have slower rate of the spoilage than other milk due to the low rate of lactobacilli reproduction. That means that the milk stored in the temperature of 1˚C is expected to have slower decrease in its pH.

Background Information

Knowing the best temperature for milk storage is important since it can either cause milk spoilage or extend it freshness. At home, my grandmother tends to store milk outdoors while the temperature outside is not high. She lives in a countryside and during colder parts of a year the electricity does not usually work. So she is left with no choice but to store milk outdoors. Moreover, my grandmother refuses to keep milk that tastes or smells 'strange' while the smell and taste of the milk might not be necessarily indicates of milk being expired. Expired milk is bad for the health because of increased number of bacteria in it while it is wasteful to throw away milk that might be still safe to drink. Therefore, I would like to investigate how long she could keep milk in different low temperatures so that I could tell her my results.

Cow milk comes to grocery stores either after being "prepared" by pasteurization, sterilization or UHT.

- Pasteurization involves milk being heated to 72˚C for 15 seconds, which kills most of the bacteria save for lactobacilli. That process eliminates many fermentative bacteria as well as pathogenic ones.
- Sterilization has milk heated to 105˚C for 15 seconds, which kills all bacteria, however, milk changes its tastes to "cooked".

- UHT milk is heated to 130°C for 2 seconds. That is enough to kill all bacteria without changing the milk flavor.

Hence, pasteurized milk can be kept in the fridge for about 10 days until it goes bad. Sterilized and UHT milk, on the other hand, do not need to stay in a fridge as long as it stays unopened inside its container. When the container is unsealed microbes can get inside the milk, which requires the milk to be stored inside a fridge. Raw milk even if kept refrigerated will go bad quickly (sooner that any of "prepared" milk) because of the presence of psychrophilic bacteria that is cold-tolerant ("Microbes and Food. We Are What We Eat. Milk." *Microbes and Food. We Are What We Eat. Milk*).

Lactose (disaccharide) is an integral part of the milk and other diary products. Milk also contains lactic acid bacteria (*lactobacillus* genus) that converts lactose into glucose and then lactic acid. Therefore, the pH of the milk is a good indicator of determining the milk quality. Milk pH stands between 6.8 and 6.5. When milk becomes spoiled the pH goes down thus increasing the acidity of the milk due to the bacteria activity. When milk pH goes below 6.5 it starts getting spoiled ("Measuring the Ph of Milk." *SlideShare*). Lactobacilli continues producing lactic acid until milk pH reached 4.6 since after that point bacteria die out.

Growth/reproduction of bacteria is controlled by cooling the milk; it will not kill bacteria but slow the reproduction rate down greatly ("Bacteria." *Microbiology*). There is an optimum temperature at which bacteria grows the most. Temperatures below the optimum one slow down bacterial growth or stop it whatsoever without killing bacteria. However, if temperatures are above optimum it starts killing bacteria.

The pH is measured using a pH sensor or a traditional pH meter ("Measuring the Ph of Milk." *SlideShare*).

Design

Variables & Controls

Independent variables: Temperature of milk storage

Dependent variable: change in pH of milk

Factors to be controlled	Impact on data if not controlled	How it will be controlled
Same brand of milk	Different brands of milk might have different composition of milk with different amount of lacto bacteria	I will buy the same kind of milk
All of milk pasteurized	This process kills most of the bacteria save for lactobacilli	Look at the milk description

Same amount of milk in beakers	Different amount of milk might affect the rate at which milk will go bad	I will put 60 ml of milk in each beaker using a measuring cylinder
Time period on which pH sensor is placed inside a cup.	It takes time for pH sensor to reach the value of pH so that it does not fluctuate	Let the pH sensor be inside a cup for a minute

Materials

Paper cups (25)
Plastic lids (25)
Measuring cylinder (100 ml)
pH Meter (*Science Workshop*™ 500 Interface)
Laptop with PASCO Capstone app
Buffer Solution pH 10
Buffer Solution pH 4
Buffer Solution pH 7
Beaker (100 ml)
Distilled water
Fridge at 1˚C
Fridge at 5˚C
Fridge at 7˚C

Procedure

1. Pour 60 ml of milk in a paper cup using measuring cylinder
2. Set the pH meter
3. Plug it in the laptop
4. Open PASCO Capstone app
5. Go to "Hardware Setup"
6. Choose a yellow circle which the wire is plugged to
7. Add pH sensor
8. Go to calibration
9. Choose pH as a type of measurement to calibrate
10. Go to "next"
11. Choose "Two Standards" as a type of calibration you would like to perform
12. Press "next"
13. For the first point "Standard Value" put "4.00"
14. Put pH sensor into the Buffer Solution with pH of 4
15. When the value gets still press "Set Current Value to Standard Value"

16. For the second point "Standard Value" put 10.0
17. Rinse the pH sensor with distilled water
18. Put pH sensor into the Buffer Solution with pH of 10
19. When the value gets still press "Set Current Value to Standard Value"
20. Rinse the pH sensor with distilled water
21. Choose "Digits" on the right side of the page to display data
22. Press on "select measurement" and choose "pH"
23. Press "Record"
24. Put pH sensor into the Buffer Solution with pH of 7 to check the calibration
25. If the digits show 7.0 rinse the pH sensor with distilled water
26. Pour 60 ml of milk in each of 25 cups and label them
27. Put the pH sensor into the milk
28. Measure pH of milk in each cup
29. Record the data
30. Pour the rest of the milk (60 ml) into 20 paper cups
31. Close them with lids
32. Place the first 5 cups in a fridge with temperature of 1°C
33. Place the second 5 cups in a fridge with temperature of 5°C
34. Place the third 5 cups in a fridge with temperature of 7°C
35. Place the fourth 5 cups in an area at 10°C
36. Place the fifth 5 cups in a room temperature as a control.
37. Leave all 25 cups for 11 days overall for each cup
38. Take the pH on the 3rd day
39. Record the data
40. Check on milk pH on the 5th day
41. Record the data
42. Check on milk pH on the 7th day
43. Record the data
44. Check on milk pH on the 9th day
45. Record the data
46. Check on milk pH on the 11th day
47. Record the data
48. Plot graphs
49. Compare the data
50. Draw a conclusion

Safety: beware Buffer solutions with pH of 4 and 10. If some got on the hands rinse them with distilled water. Wear safety googles.

Data Collection and Processing

Milk Spoilage Experiment Data Table 1 (Change in pH of milk stored at different temperatures)

1st Day pH (±0.1)

Temperature (˚C)	1st Trial	2nd Trial	3rd Trial	4th Trial	5th Trial	Average
1	7.0	7.0	6.9	7.0	7.0	7.0
5	6.9	7.0	7.0	7.0	7.0	7.0
7	6.9	6.9	7.0	7.0	7.0	7.0
10	7.0	7.0	7.0	7.0	7.0	7.0
20	7.0	6.9	7.0	7.1	7.0	7.0

3rd Day pH (±0.1)

Temperature (˚C)	1st Trial	2nd Trial	3rd Trial	4th Trial	5th Trial	Average
1	6.9	7.0	7.0	7.0	7.0	7.0
5	7.0	7.0	6.9	7.0	7.0	7.0
7	6.9	6.9	7.0	7.0	7.0	7.0
10	7.0	7.0	7.0	7.0	7.0	7.0
20	7.0	6.9	7.0	7.0	7.0	7.0

5th Day (±0.1)

Temperature (˚C)	1st Trial	2nd Trial	3rd Trial	4th Trial	5th Trial	Avarage
1	6.9	7.0	7.0	7.0	7.0	7.0
5	7.0	7.0	6.9	7.0	7.0	7.0
7	6.9	6.9	7.0	7.0	7.0	7.0
10	7.0	6.9	7.0	7.0	7.0	7.0
20	6.3	6.1	6.2	5.7	5.8	5.9

7th Day pH (±0.1)

Temperature (˚C)	1st Trial	2nd Trial	3rd Trial	4th Trial	5th Trial	Average
1	6.9	7.0	7.0	7.0	7.0	7.0
5	7.0	7.0	6.9	7.0	7.0	7.0
7	6.9	6.8	7.0	7.0	7.0	7.0
10	6.8	6.8	6.7	6.8	6.8	6.8
20	6.0	5.9	6.1	5.5	5.6	5.8

9th Day (±0.1)

Temperature (˚C)	1st Trial	2nd Trial	3rd Trial	4th Trial	5th Trial	Average
1	6.9	7.0	7.0	7.0	7.0	7.0
5	6.9	7.0	6.9	6.9	6.9	6.9
7	6.8	6.8	6.8	6.8	6.9	6.8
10	6.4	6.5	6.4	6.3	6.4	6.4
20	6.0	5.9	5.8	5.5	5.4	5.7

11th Day (±0.1)

Temperature (˚C)	1st Trial	2nd Trial	3rd Trial	4th Trial	5th Trial	Average
1	6.9	7.0	7.0	7.0	7.0	7.0
5	6.9	7.0	6.9	6.9	6.9	6.9
7	6.8	6.7	6.8	6.8	6.9	6.8
10	6.3	6.2	6.2	6.2	6.0	6.2
20	5.9	5.8	5.6	5.5	6.0	5.6

Average Change of Milk pH after 11 days Data Table 2

Temperature (˚C)	Average Change of Milk pH (1 dp) (±0.1)
1	0.0
5	0.1
7	0.2
10	0.8
20	1.4

Average = Sum of values/ Number of values

E.g. Average = (6.0 + 5.9 + 5.8 + 5.5 + 5.4)/5 = 6.7 (1 dp)

Change of Milk pH = 1st day pH – 11th day pH

E.g. Change of 20˚C Milk pH = 7.0 – 5.6 = 1.4

Milk Spoilage Experiment Data Table 2 (Qualitative Data)

1st Day pH (±0.1)

Temperature (˚C)	1st Trial	2nd Trial	3rd Trial	4th Trial	5th Trial
1	White, no smell	White, no smell	White, no smell	White, no smell	White, no smell
5	White, no smell	White, no smell	White, no smell	White, no smell	White, no smell
7	White, no smell	White, no smell	White, no smell	White, no smell	White, no smell
10	White, no smell	White, no smell	White, no smell	White, no smell	White, no smell
20	White, no smell	White, no smell	White, no smell	White, no smell	White, no smell

3rd Day pH (±0.1)

Temperature (˚C)	1st Trial	2nd Trial	3rd Trial	4th Trial	5th Trial
1	Half frozen, no smell	White, no smell	Half frozen, no smell	White, no smell	White, no smell
5	White, no smell	White, no smell	White, no smell	White, no smell	White, no smell
7	White, no smell	White, no smell	White, no smell	White, no smell	White, no smell
10	White, no smell	White, no smell	White, no smell	White, no smell	White, no smell
20	White, no smell	White, no smell	White, no smell	White, no smell	White, no smell

5th Day (±0.1)

Temperature (˚C)	1st Trial	2nd Trial	3rd Trial	4th Trial	5th Trial
1	Half frozen, no smell	Half frozen, no smell	Half frozen, no smell	White, no smell	White, no smell
5	White, no smell	White, no smell	White, no smell	White, no smell	White, no smell
7	White, no smell	White, no smell	White, no smell	White, no smell	White, no smell
10	White, no smell	White, no smell	White, no smell	White, no smell	White, no smell
20	White, unpleasant/sour smell	White, unpleasant/sour smell	White, unpleasant/sour smell	White, unpleasant/sour smell	White, unpleasant/sour smell

7th Day pH (±0.1)

Temperature (°C)	1st Trial	2nd Trial	3rd Trial	4th Trial	5th Trial
1	Half frozen, no smell	Half frozen, no smell	Half frozen, no smell	White, no smell	White, no smell
5	White, no smell	White, no smell	White, no smell	White, no smell	White, no smell
7	White, no smell	White, no smell	White, no smell	White, no smell	White, no smell
10	White, no smell	White, no smell	White, no smell	White, no smell	White, no smell
20	White, unpleasant/sour smell	White, unpleasant/sour smell	White, unpleasant/sour smell	White, unpleasant/sour smell	White, unpleasant/sour smell

9th Day

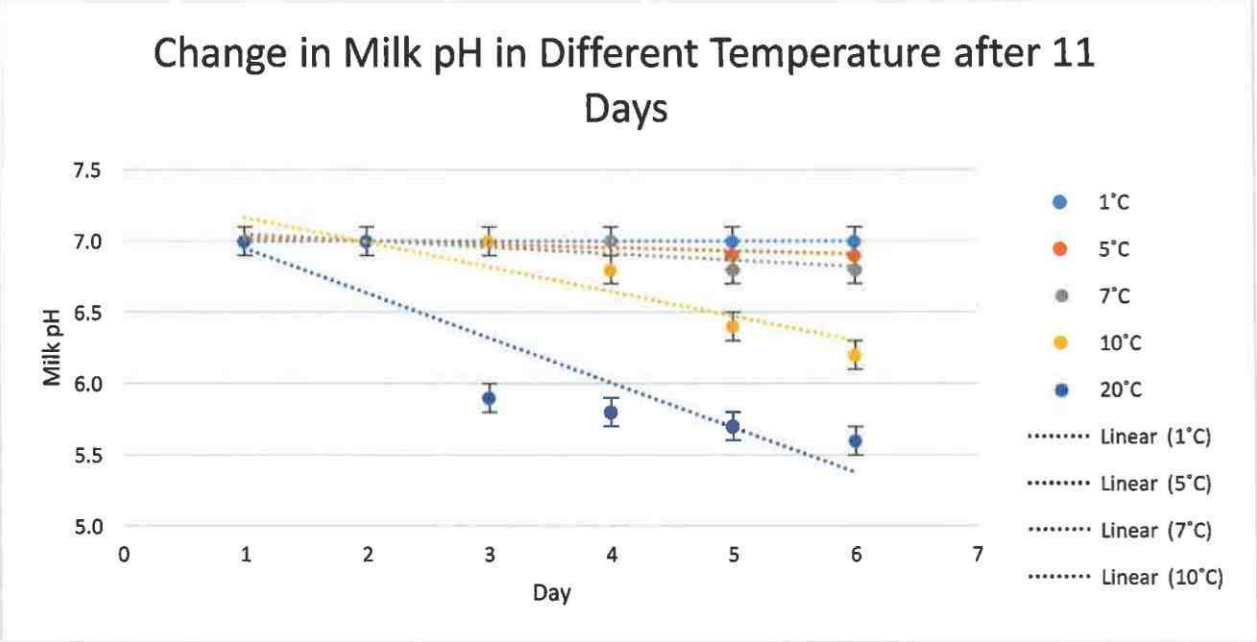
Temperature (°C)	1st Trial	2nd Trial	3rd Trial	4th Trial	5th Trial
1	Half frozen, no smell	Half frozen, no smell	White, no smell	White, no smell	White, no smell
5	White, no smell	White, no smell	White, no smell	White, no smell	White, no smell
7	White, no smell	White, no smell	White, no smell	White, no smell	White, no smell
10	White, unpleasant/sour smell	White, unpleasant/sour smell	White, unpleasant/sour smell	White, unpleasant/sour smell	White, unpleasant/sour smell
20	Milk curdles, pale-yellow fluid, unpleasant/sour smell	Milk curdled, pale-yellow fluid, unpleasant/sour smell	Milk curdled, pale-yellow fluid, unpleasant/sour smell	Milk curdled, pale-yellow fluid, unpleasant/sour smell	Milk curdled, pale-yellow fluid, unpleasant/sour smell

11th Day (±0.1)

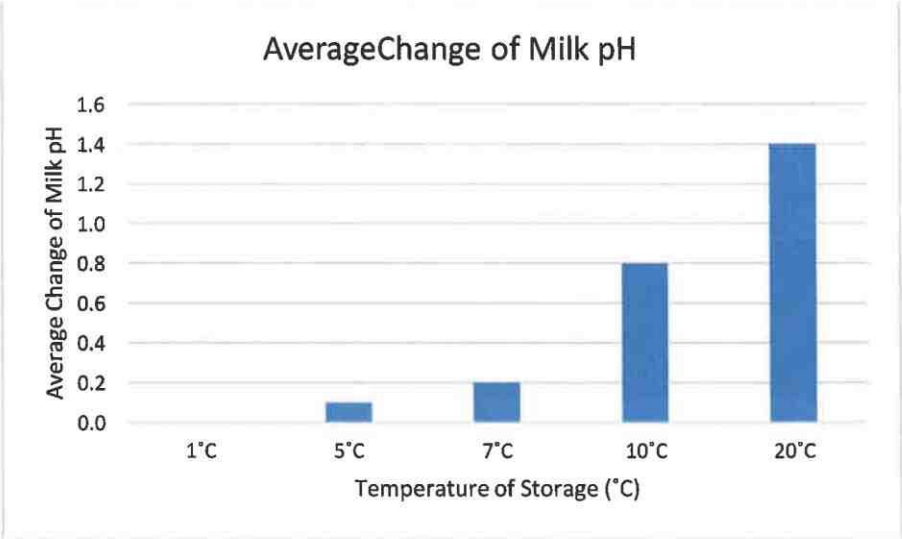
Temperature (°C)	1st Trial	2nd Trial	3rd Trial	4th Trial	5th Trial
1	Half frozen, no smell	Half frozen, no smell	Half frozen, no smell	White, no smell	White, no smell
5	White, no smell	White, no smell	White, no smell	White, no smell	White, no smell
7	White, no smell	White, no smell	White, no smell	White, no smell	White, no smell
10	Milk curdles, pale-yellow fluid, unpleasant/sour smell	Milk curdled, pale-yellow fluid, unpleasant/sour smell	Milk curdled, pale-yellow fluid, unpleasant/sour smell	Milk curdled, pale-yellow fluid, unpleasant/sour smell	Milk curdled, pale-yellow fluid, unpleasant/sour smell
20	Milk curdles, pale-yellow fluid, unpleasant/sour smell	Milk curdled, pale-yellow fluid, unpleasant/sour smell	Milk curdled, pale-yellow fluid, unpleasant/sour smell	Milk curdled, pale-yellow fluid, unpleasant/sour smell	Milk curdled, pale-yellow fluid, unpleasant/sour smell

Data Analysis

Graph 1



Graph 2



The graphs and average data tables show that pH of milk stored in the room temperature gradually decreased by 1.4 during 11 days after measuring the initial pH. The error bar is set on ± 0.1 since it was the value on which the values on pH sensor were fluctuating. The pH of milk kept at 10˚C gradually decreased by 0.8. The line of best fit shows the downward trend. Moreover, the pH of milk placed in the fridge at 7˚C gradually decreased by 0.2. The line of best fit shows the downward trend as well. As for pH of milk store at 5˚C, it gradually decreased by 0.1. In addition, pH of milk stored in 1˚C has not changed. The line of best fit is parallel to the x-axis. The overall decrease in pH of milk placed in 1˚C, 5˚C and 7˚C is not significant since the values 6.9 and 6.8 are close to the neutral point (Image 1: pH scale below), which indicates that the activity of lactobacilli was insignificant compared to the change in 10˚C and 20˚C where milk became more significantly acidic. Also pH of 6.9 and 6.8 are larger than 6.5, which is considered to be point when milk expires ("Measuring the Ph of Milk." *SlideShare*). Also by looking at the Milk Spoilage Experiment Data Table 1 it is evident that the initial pH of the milk was above predicted 6.7, and so another brand of milk pH was checked, which turned out to be 7.0 as well, which indicates that pH of 7.0 is a normal pH of distilled milk sold in grocery stores.

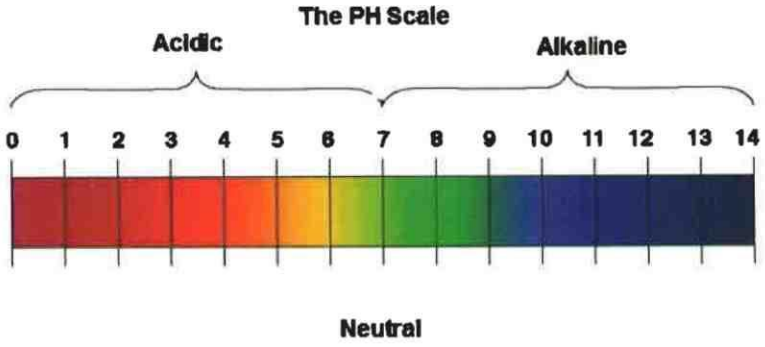


Image 1 ("Alkaline Without Alkalinity." *Dr. Sircus.* 17 Sept. 2014. Web. 24 Oct. 2015.)

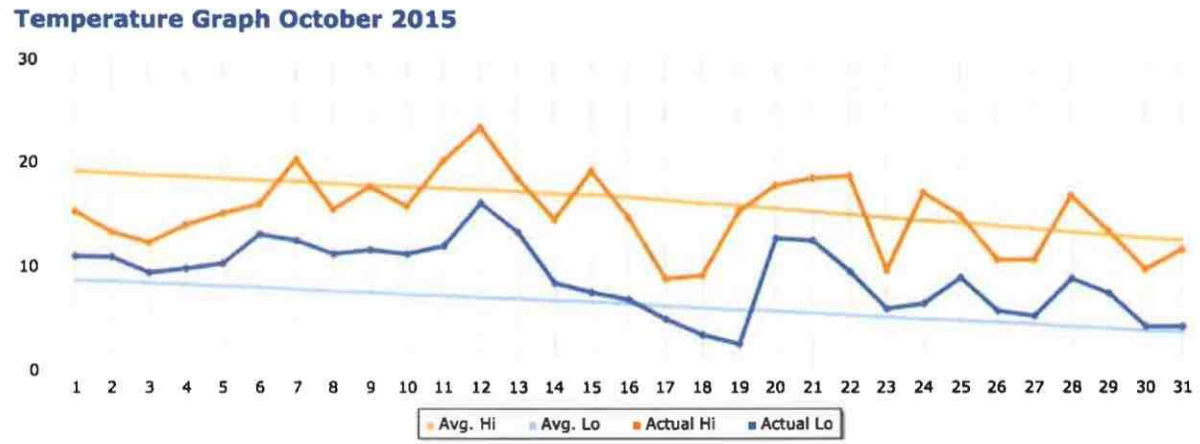
Conclusion and Evaluation

Conclusion

The hypothesis stated that the milk stored in the temperature of 1˚C is expected to have slower decrease in its pH due to the lower rate of lactobacilli reproduction. The processed data and the graph helped support and prove it since the pH of milk stored in 1˚C has not changed in 11 days the experiment has been conducted. However, the qualitative data table showed that some of milk stored in 1˚C got half frozen, which makes this temperature not very suitable for storing milk since some time will be needed for milk to turn back to liquid. The data and the graph displayed that the milk spoilage did not occur in temperatures 5˚C and 7˚C either since the change in pH were 0.1 and 0.2 respectively.

That suggests that the lactobacilli reproduction is greatly hindered by the range of temperature from 1˚C to 7˚C. That means that milk can be stored in either of those temperatures. In addition, the milk stored outdoors has undergone later and lesser decrease in pH than the milk stored in room temperature, which implies that milk can be also stored outside for no more than 7 days if the temperatures are similar to ones during the experiment (chart 1 below; days experiment was conducted: from 17th October to 27th October(inclusively)). The data related to the room temperature showed that milk cannot be stored in that condition for longer than 3 days since the pH starts decreasing significantly to 5.6 after 3 days, which indicates the milk spoilage. Also the line of best fit suggests that milk pH goes below 6.5 on 2.5 day. To sum up, the temperature in which milk is stored does affect the rate of pH decrease.

Chart 1 ("St. Catharines, CA." *AccuWeather.*)



Evaluation and Improvement Methods:

The weaknesses and limitations:

Weaknesses & Strengths	How weaknesses/strengths impacted the data	Suggestions for improvement
Milk pH was not measured daily during 11 days.	The changes in pH have been rather abrupt and gradual change was not fully shown. The need of calibration for each day might have cause errors in measuring pH of milk.	Measure milk pH everyday or even twice or three times per day.

Work Cited

"Bacteria." *Microbiology*. Web. 25 Oct. 2015.

"Consequences of Drinking Expired Milk." *LIVESTRONG.COM*. LIVESTRONG.COM, 13 Apr. 2015. Web. 7 Nov. 2015.

Lu, Michael, Yvonne Shiau, Jacklyn Wong, Raishay Lin, Hannah Kravis, Thomas Blackmon, Tanya Pakzad, Tiffany Jen, Amy Cheng, Jonathan Chang, Erin Ong, Nima Sarfaraz, and Nam Sun Wang. "Milk Spoilage: Methods and Practices of Detecting Milk Quality." FNS Food and Nutrition Sciences 04.07 (2013): 113-23. Web. 28 Sept. 2015.

"Measuring the Ph of Milk." *SlideShare*. N.p., n.d. Web. 28 Sept. 2015. <http://www.slideshare.net/farhana25/measuring-the-ph-of-milk>.

"Microbes and Food. We Are What We Eat. Milk." *Microbes and Food. We Are What We Eat. Milk*. N.p., n.d. Web. 28 Sept. 2015. <http://resources.schoolscience.co.uk/SGM/sgmfoods12.html>.

"St. Catharines, CA." *AccuWeather*. Web. 8 Nov. 2015.

The experiment was conducted only for 11 days while not reaching the expiry date of the milk (October 30th, 2015) due to restricted amount of time available.	Unable to observe which of milk stored in 1˚C, 5˚C and 7˚C would expire first. That hindered determining which of temperatures is best for milk storage.	Milk pH should have been continued being measured even after expiry date to observe which of temperatures would keep milk fresh for longer
Only one package of milk was tested which limits the reliability of the experiment.	Best storage temperature might be different for each brand of milk. While it is safe to keep milk outside for 7 days for one kind of brand it might be different for another.	More trials could have been done with different brands or packages of milk so that the trend was applicable to wider range of milk. Also the use of different types of milk such as soy milk, goat milk, rice milk, almond milk and cow milk would have enriched the experiment. In addition, Use of pasteurized, sterilized and UHT milk would have shown the difference in spoilage of those kinds of milk.
Some cups in room temperature might have been placed closer to some sort of heat; some cups in outdoors temperature might have been exposed to the sun, which would have affected the temperature of milk.	Cups placed in the same place might have had different temperatures thus different rate of bacteria growth.	Make sure that cups are placed on the same distance from the source of either cold or heat.

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For first use in 2016 examinations

4/ICCS

Group 4: Individual candidate cover sheet (Biology, Chemistry and Physics)

Arrival date: Session:

School number: School name:

- Complete this form in the working language of your school (English, French, Spanish).
- The form must be completed by the teacher and candidate.
- A completed copy should be retained by the school.

Subject: Biology Level: HL

Candidate name: Session number:

Candidate section:
To be completed by the candidate.

Title of the group 4 project: Building a Rube Goldberg Machine

Write a reflective statement of about 50 words outlining your involvement in the group 4 project:

During this Science Project, I worked with my friends, which made it quite enjoyable for me. Even though we faced many problems during the creation of our mechanism I think this was a great experience for all of us. Our mechanism represented a blood circulation in human body, precisely, a gas exchange from CO2 to O2. My part represented how carbon dioxide (a tennis ball) is replaced by oxygen in lungs (bags). My part depended on whether the fan will be switched on by another ball before, which was quite tricky and we also needed enough energy to enable the tennis ball hit a heavier ball, which required all of us think over my part. Overall, I feel that we all worked hard and I really appreciate the effort everyone put in our project.

Title of individual investigation: Cow Milk Spoilage in Five Different temperatures

Candidate declaration: I confirm that this investigation is my own work and is the final version. I have acknowledged each use of words or ideas of another person, whether written, oral or visual.

Candidate's signature: Date:

Turn over