

An Investigation on the effect of Sucrose Concentration Levels (0%, 5%, 10%, 20%, 30%, 50%) on the pH of Pura Original Full Cream Milk over a duration of 143 hours.

Introduction:

Milk is known as an excellent source of protein with essential nutrients including calcium, potassium, magnesium, vitamin A and vitamin B-12 (Rogelj & Irena, 2000). Given its benefits to health, milk is indispensable in my diet and forms my staple breakfast every morning. Nevertheless, despite the established benefits of milk consumption, milk has a limited shelf life, lasting five to six days when stored within the refrigerator and two hours in room temperature (U.S Dairy, 2017). Due to its short shelf life, the expiry date often surpasses the rate in which it is consumed, leading to massive milk wastage. This stimulated my interest to find a way to extend the expiry date of milk.

In finding a method to extend the shelf life of milk, why milk spoilage occurs needs to be understood first. Milk spoilage, which is when milk passes its expiry date and sours, occurs from the fermentation of lactose by lactic acid bacteria (Bozoglu, 2016). The raw milk obtained from cows contains microorganisms (Bozoglu, 2016). Pasteurization is an imperative procedure in treating raw milk by applying high temperature to kill pathogens and most microbes within milk to ensure its safety consumption (Dhotre, 2014). *Lactobacillus* is a heat resistant genus bacteria found in raw milk which do not perish during milk pasteurization (Bluma, 2017). As milk is stored overtime, *Lactobacillus* undergoes fermentation by metabolizing lactose and releasing lactic acid as by-product. The lactic acid lowers the pH of milk, develops milk curdle and creates a noxious smell in milk to signal that the milk is spoiled (Widyastuti, Rohmatussolihat & Febrisiantosa, 2014).

I learnt that sugar is often added to canned food and dairy products as a preservative (Rawat, 2015). This is because when excessive sugar is introduced into food medium, sugar creates a high solute concentration outside the bacterial cell in contrast to inside, establishing a hypertonic environment which results in cell to lose water by osmosis and eventually die (Sehgal, n.d.). Since lactic acid is produced by *Lactobacillus* during fermentation, if *Lactobacillus* dies in hypertonic environment, lactic acid won't be created. Consequently, the pH of milk won't reduce, and milk spoilage can be prevented. In my investigation, I am exploring whether adding sucrose, a commonly used sugar additive, will prolong the duration of milk. If sugar does demonstrate inhibitory effects on *Lactobacillus*, by adding sucrose to milk I will be able to store milk for greater periods and potentially extend the prescribed expiry date. This investigation is meaningful to me as it would help to avoid milk wastage.

Research question:

How do different levels of sucrose concentration (0%, 5%, 10%, 20%, 30%, 50%) affect the pH of full cream milk stored in 25°C over a duration of 143 hours?

Hypothesis:

H₁: As sucrose concentration level (0%, 5%, 10%, 20%, 30%, 50%) increases, it would take longer duration for the pH of milk to decrease. Previous research demonstrates high concentration of sugar inhibits bacterial growth (Mizzi, Maniscalco, Gaspari, & Chatzitzika, 2020). Therefore, as sugar concentration increases, there will be greater inhibitory effect on bacteria within milk which reduces the lowering of milk's pH overtime.

H₀: The difference in sucrose concentration (0%, 5%, 10%, 20%, 30%, 50%) does not slow the rate which the pH of milk decreases, but all sucrose concentration decreases at the same rate over 142 hours.

Independent variable:

Levels of sucrose concentration (0%, 5%, 10%, 20%, 30%, 50%)

The milk sucrose concentrations levels are made by adding granulated sucrose to milk. The sucrose concentration level is calculated by dividing the mass (g) of sucrose by the volume (ml) of milk.

$$\text{Sucrose concentration level} = \frac{\text{The gram of sucrose}}{\text{The volumn of milk}} \times 100$$

The mass of sucrose is measured on a three decimal electric balance. Five repeats are made for each level.

Sucrose sugar is selected as the independent variable because it is commercially accessible at an affordable expense. The range was selected based on my pilot study. It was demonstrated in pilot study that increasing sucrose concentration (0%, 5%, 10%, 20%, 30%, 50%) incrementally decreased bacterial colonization on nutrient agar mediums.

Dependent variable:

The pH of the milk

The pH of the milk is used to assess whether the milk has spoiled or not. Unspoiled milk has an approximate pH of 6.7 (Lu, Michael & Shiau et al. 2013). At low pH range of 4.0 – 5.0, lactic acid bacteria are capable of growing to produce lactic acid. (Lu et al., 2013) Hence, milk pH of around 4.0 indicates that the milk has spoiled.

The pH of milk is determined using a Pasco pH sensor with an uncertainty of (± 0.10). The pH of milk is controlled by stirring the head of the probe in milk until the pH reading on the Pasco sensor screen is stable to be recorded.

Risk Assessment

Table 1: Risk assessment

| <u>Hazard</u> | <u>Potential Risk</u> | <u>Safety measures</u> |
|-----------------------|--|---|
| Milk | Some students may be allergic to milk. | Store milk in the refrigerator. Do not drink in class. |
| Milk | Swallowing of expired milk may cause food poisoning and abdominal pain. (Hill, 2019) | Wear safety goggles and lab coat. Wash hand thoroughly and use hand sanitizer after handling of milk. Do not drink milk in class due to possible contamination. |
| Smell of spoiled milk | Long term exposure to the sour smell of milk may cause headaches and create nausea. (Hill, 2019) | Wear mask when handling spoiled milk. Maintain five meters from everyone else within the laboratory. |
| Glass beaker | Breakage of beaker may cut into skin. | Discard cracked beakers by sweeping up glass fragments using a brush and dustpan and dispose into nearby bin |

Controlled variables:

Table 2: Controlled variables

| <u>Controlled Variable</u> | <u>Reason of Control</u> | <u>Method of control</u> |
|---|--|--|
| Type of milk used | Different variety of milk may contain different substance. For example, favour added milk may contain sweet additives which impacts the activity of bacteria. | The same type of milk (full cream) and brand of milk (Pura Original) is used. |
| Volume of milk added to each beaker | If the volume of milk added increases, there will be more lactose present for metabolization, which would impact the result by potentially increasing the rate which the pH decreases. | The same volume of milk, 25ml, is measured using a 100ml measuring cylinder (± 0.01 ml) for all solutions. |
| Volume of sucrose | Larger volume of sucrose would take longer time to dissolve in milk or may not dissolve at all. | The sucrose is granulated to help it dissolve in milk |
| Temperature of milk storage | A high temperature is likely to facilitate the growth of <i>Lactobacillus</i> while a low temperature may stimulate the bacteria to go into dormancy. (Jones & Lennon, 2010) | The milk sucrose concentrations levels (milk solutions) were placed within an incubator at 25°C to facilitate activity of <i>Lactobacillus</i> . Temperature 25°C is identified to be the right temperature for the growth of <i>Lactobacillus</i> . |
| The duration of milk solution stored within the incubator | Longer duration of milk storage would mean that more fermentation is occurring, indicating more lactic acid is produced which leads to faster decrease in milk pH level. | All milk solutions are extracted from the incubator for testing of pH at regulated time: 27, 43, 73, 142 hours. |
| The same Pasco pH sensor utilization | Each Pasco pH sensor is calibrated differently. Utilization of different pH sensor would produce a difference in pH reading. | The Pasco pH sensor used on day one was marked by a yellow sticker on the side for distinguishing and is repeatedly used for all pH testing. |

Ethical concerns:

- Milk should be bought from a trusted company that acquires milk through ethical treatment of cows. The milk used in this experiment is bought from Pura Original Milk, a well-known Australia brand. However, Pura Original Milk does state how the company obtains milk, therefore, it is acknowledged that maltreatment of cows to obtain milk may be an underlying issue.
- The use of milk within the experiment was minimized to prevent wastage of milk. The milk was minimized by limiting to five replications for each sucrose concentration level, and minimal use of milk in each replication (25ml).

Materials:

- 30 x 50ml beaker (± 0.5 ml)
- 100ml measuring cylinder (± 0.1 ml)
- 150g of granulated sucrose (Natural Sweetener)
- 1L of distilled water
- 1 box of tissue
- Milk - Full cream (Pura Original)
- Pasco pH sensor (± 0.10)
- Electric balance (± 0.001 g)
- 1 x plastic spoon
- 6 x sterile spatula
- 20cm x 30cm rectangular container

Aseptic technique:

Aseptic technique was maintained throughout the experiment to prevent contamination with the milk.

To apply aseptic technique:

- All spatula used were sterilised prior usage for 4 minutes at 121°C at 12 PSI using an electrical steam autoclave.
- 70% ethanol was sprayed onto the laboratory bench to sterilize the surface of the working area.

Method:

1. Separation of milk into smaller containers

1. 30 x 50ml beakers are separated into 6 by 5 grid - into 6 rows and 5 columns. Each row of beaker is labelled A, B, C, D, E from left to right in alphabetical order by a blue maker to show repeating trials.
2. Each column of beaker is labelled 0%, 5%, 10%, 20%, 30%, 50% in order from top to bottom by a blue maker to show levels of sucrose concentration.
3. 25ml of milk from Pura Original is measured using a 100ml measuring cylinder and added into each beaker.

2. Adding sugar to milk

Sucrose is added into each beaker according to the following table:

Table 3: Measurement of sucrose concentration level in proportion to milk volume and sucrose mass

| Sucrose concentration level (%) | Volume of milk (ml) (± 0.1 ml) | Mass of sucrose (g) (± 0.001 g) |
|---------------------------------|-------------------------------------|--------------------------------------|
| 0 | 25.0 | 0 |
| 5 | 25.0 | 1.250 |
| 10 | 25.0 | 2.500 |
| 20 | 25.0 | 5.000 |
| 30 | 25.0 | 7.500 |
| 50 | 25.0 | 12.500 |

1. A 50ml empty beaker is placed on a measuring balance and the mass was set to zero.
2. Granulated sucrose is added into the empty beaker until the mass of sucrose reached the mass outlined in table 2 above.
3. Granulated sucrose is added into the corresponding milk beaker from the beaker used to hold granulated sucrose.
4. A sterile spatula is used to stir the sucrose added milk (milk solution) for 30 seconds to help the sucrose dissolve.
5. Repeat steps 1-4 for all solutions while disposing used spatula and using a new sterile spatula for each different concentration level of sucrose.

3. Incubation of milk

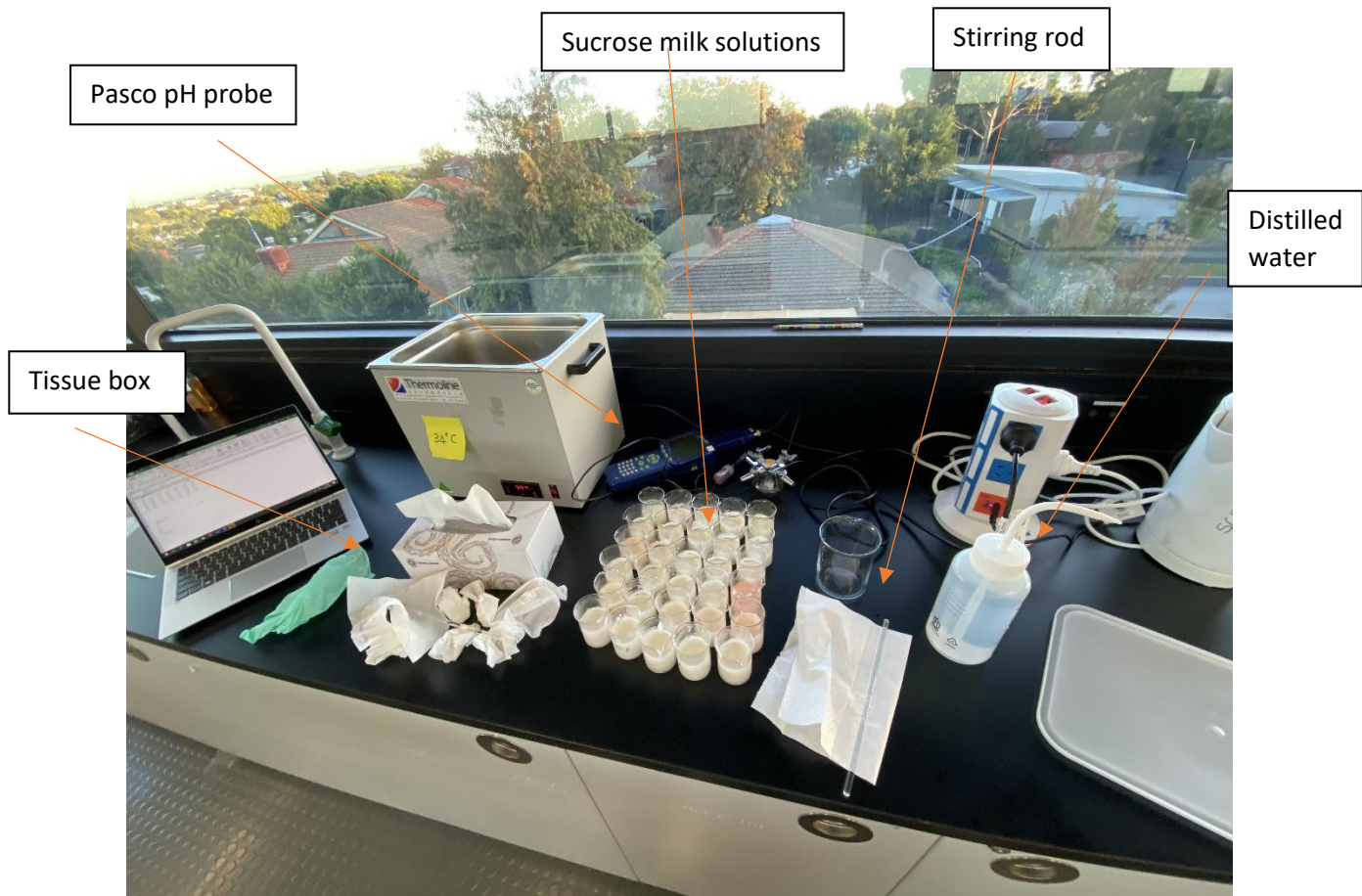
1. All beakers are placed into a container with lid closed and stored in an incubator at 25°C

4. Testing of pH

1. The beakers are placed on the bench in 6 by 5 grid (6 rows by 5 columns). Rows are placed from left to right in alphabetical order and columns from top to the bottom in increasing sucrose concentration.
2. A stirring rod is used to stir milk solutions for 10 seconds
3. The head of pH probe is placed into each milk solution
4. The pH probe is stirred until the pH reading is stable, and then recorded in an excel sheet on laptop

5. The head of the pH probe is gently rinsed with water to dispose remnant milk from last trial
6. The head of the pH probe is gentle rubbed with a tissue to dry
7. Repeat steps 1-6 for each milk solution

Annotated photograph of experimental process



Qualitative data

Table 4: Qualitative observations

| | |
|---------------------------|--|
| When fresh milk is poured | Milk solutions are fully fluid in white colour. |
| 27 hours | Milk solutions with sucrose concentration level below 30% concentration have formed coagulated lumps at the surface. The bottom half of milk solutions became translucent. |
| 43 hours | Some of the milk solutions have developed orange and pink colours. Curdling is shown on the surface of all milk solutions below 30%. When stirred with the stirring rod, the milk solution is thick and viscous. A pungent, sour smell is emitted from the milk. |
| 73 hours | Curdling has formed on all surface of milk solutions. 0%, 5%, 10%, 20% are fluids. The texture of the milk solutions 30% and 50% are thick and lumpy. Couple of the milk solutions have a pink colour. A worsened repellent smell is emitting from the milk. |

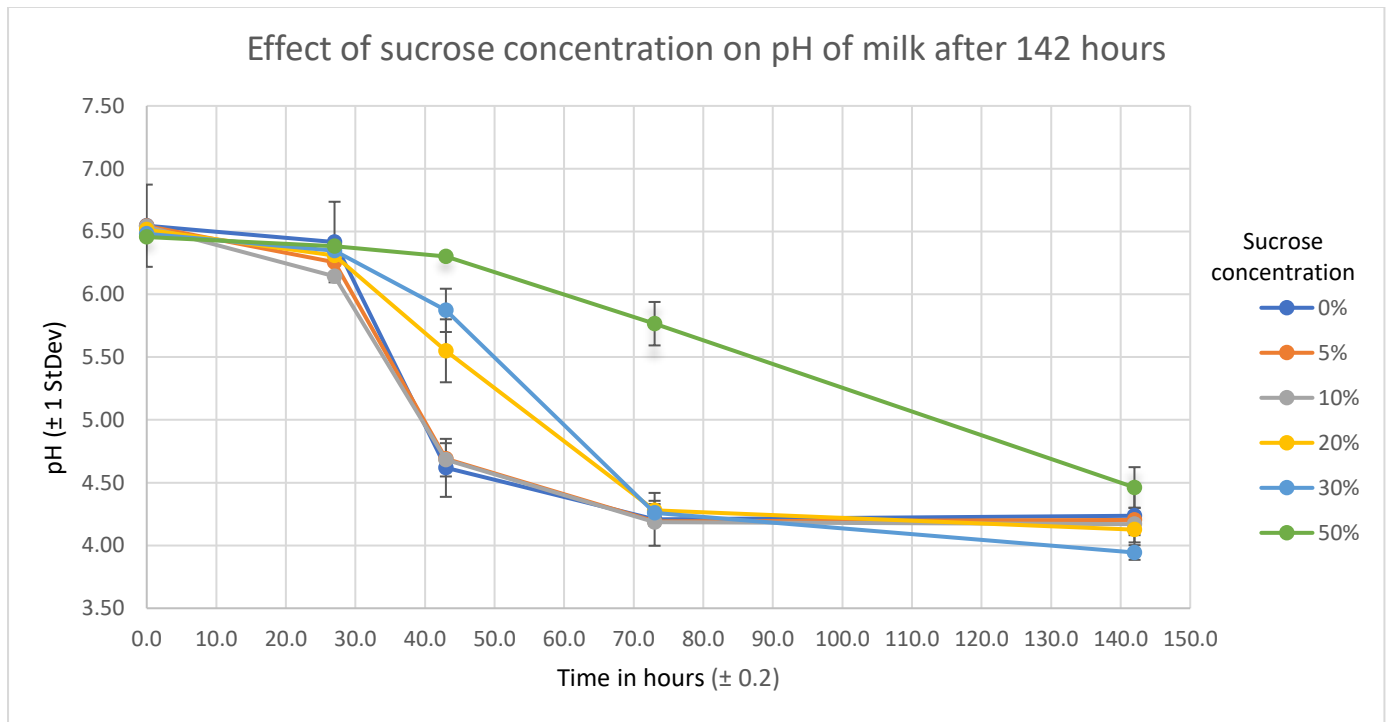
Quantitative Raw Data

Table 5: The pH (± 0.10) of varying levels of sucrose concentration (milk solution) over 5 trials over 142 hours measured using a Pasco pH sensor

| Sucrose concentration (%) | Time of duration (hours) (± 0.2) | pH (± 0.10) | | | | | pH | |
|---------------------------|--|-------------------|---------|---------|---------|---------|------|----------------------------|
| | | Trial 1 | Trial 2 | Trial 3 | Trial 4 | Trial 5 | Mean | SD (Standard deviation) |
| 0 | 0.0 | 6.54 | 6.54 | 6.54 | 6.55 | 6.56 | 6.55 | 0.01 |
| | 27.0 | 6.41 | 6.41 | 6.41 | 6.42 | 6.43 | 6.42 | 0.01 |
| | 43.0 | 4.72 | 4.50 | 4.70 | 4.75 | 4.42 | 4.62 | 0.13 |
| | 73.0 | 4.26 | 4.19 | 4.21 | 4.21 | 4.17 | 4.21 | 0.03 |
| | 142.0 | 4.37 | 4.12 | 4.23 | 4.17 | 4.29 | 4.24 | 0.09 |
| 5 | 0.0 | 6.55 | 6.54 | 6.54 | 6.54 | 6.55 | 6.54 | 0.00 |
| | 27.0 | 6.14 | 6.13 | 6.31 | 6.27 | 6.42 | 6.25 | 0.11 |
| | 43.0 | 4.70 | 4.63 | 4.56 | 4.62 | 4.94 | 4.69 | 0.13 |
| | 73.0 | 4.19 | 4.21 | 4.13 | 4.18 | 4.25 | 4.19 | 0.04 |
| | 142.0 | 4.01 | 4.30 | 4.05 | 4.35 | 4.31 | 4.20 | 0.14 |
| 10 | 0.0 | 6.54 | 6.54 | 6.54 | 6.54 | 6.54 | 6.54 | 0.00 |
| | 27.0 | 6.18 | 6.20 | 6.20 | 6.19 | 5.95 | 6.14 | 0.10 |
| | 43.0 | 4.67 | 4.55 | 4.65 | 4.70 | 4.84 | 4.68 | 0.09 |
| | 73.0 | 4.13 | 4.14 | 4.20 | 4.20 | 4.26 | 4.19 | 0.05 |
| | 142.0 | 3.92 | 3.98 | 4.20 | 4.38 | 4.37 | 4.17 | 0.19 |
| 20 | 0.0 | 6.51 | 6.52 | 6.52 | 6.51 | 6.51 | 6.51 | 0.00 |
| | 27.0 | 6.39 | 6.24 | 6.27 | 6.35 | 6.29 | 6.31 | 0.05 |
| | 43.0 | 5.58 | 5.46 | 5.79 | 5.80 | 5.12 | 5.55 | 0.25 |
| | 73.0 | 4.30 | 4.32 | 4.33 | 4.32 | 4.13 | 4.28 | 0.08 |
| | 142.0 | 4.16 | 4.00 | 4.16 | 4.41 | 3.90 | 4.13 | 0.17 |
| 30 | 0.0 | 6.48 | 6.48 | 6.48 | 6.49 | 6.48 | 6.48 | 0.00 |
| | 27.0 | 6.38 | 6.33 | 6.37 | 6.35 | 6.31 | 6.35 | 0.03 |
| | 43.0 | 6.16 | 5.83 | 5.96 | 5.71 | 5.70 | 5.87 | 0.17 |
| | 73.0 | 4.27 | 4.26 | 4.32 | 4.13 | 4.32 | 4.26 | 0.07 |
| | 142.0 | 3.89 | 4.03 | 3.98 | 3.87 | 3.95 | 3.94 | 0.06 |
| 50 | 0.0 | 6.47 | 6.45 | 6.45 | 6.45 | 6.46 | 6.46 | 0.01 |
| | 27.0 | 6.38 | 6.38 | 6.38 | 6.37 | 6.40 | 6.38 | 0.01 |
| | 43.0 | 6.31 | 6.28 | 6.32 | 6.30 | 6.29 | 6.30 | 0.01 |
| | 73.0 | 5.63 | 5.58 | 5.67 | 5.98 | 5.97 | 5.77 | 0.17 |
| | 142.0 | 4.26 | 4.34 | 4.70 | 4.59 | 4.42 | 4.46 | 0.16 |

Processed Data

Figure 1: The mean pH of each level of sucrose concentrated milk over 142 hours with standard deviation error bars

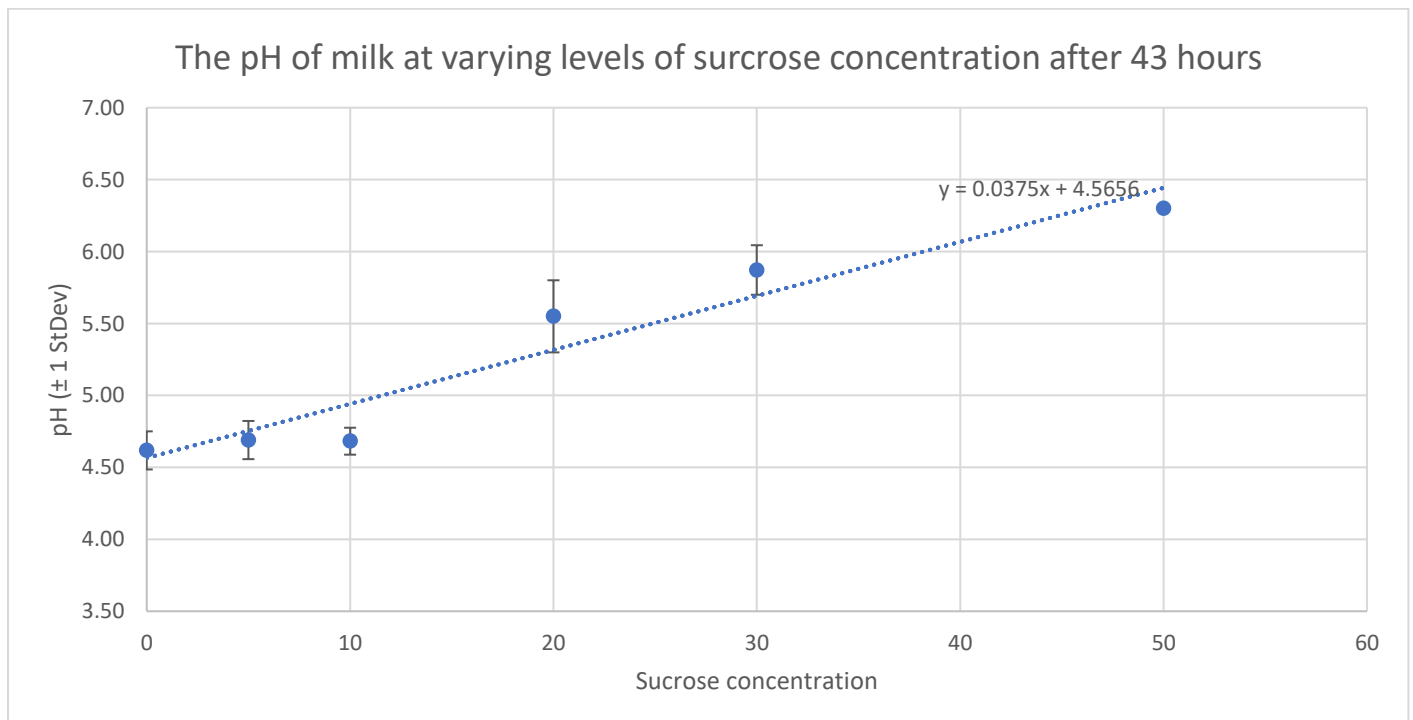


Y error bars show standard deviation of pH of milk.

X error bars show uncertainty ± 0.2 hours of the time duration.

An overall decreasing trend is seen in Figure 1. Variations of pH are observed at 43 hours, 73 hours, and lastly 142 hours. Data is extrapolated at these hour intervals and used in the creation of Figure 2, 3, 4 to analyse the significance of relationship between sucrose concentration levels and pH of milk.

Figure 2: The mean pH of milk sucrose concentration levels at the 43 hours interval with standard deviation error bars

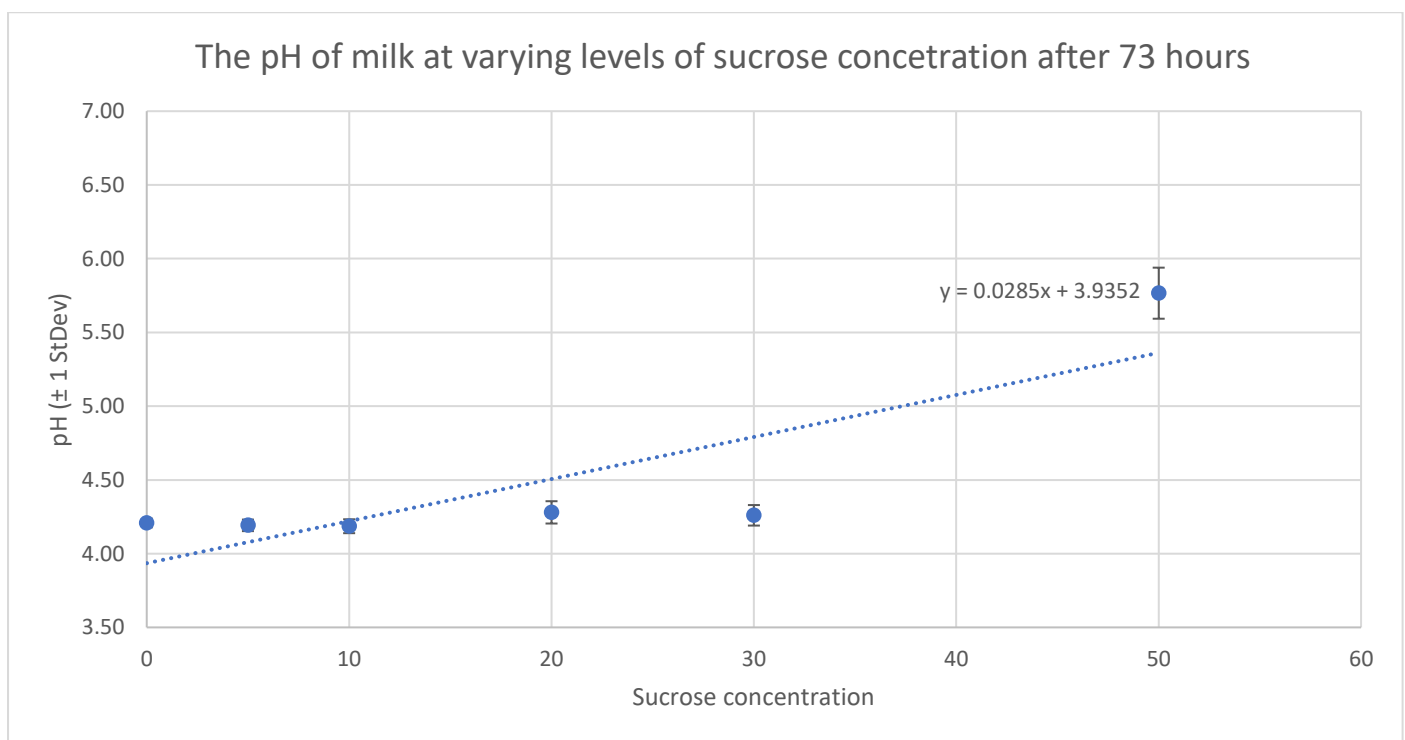


One way ANOVA statistical test

| Anova: Single Factor | | | | | | |
|----------------------|-------------|--------|-------------|----------|---------|---------|
| SUMMARY | | | | | | |
| Groups | Count | Sum | Average | Variance | | |
| 0 | 5 | 115 | 23 | 320 | | |
| 4.618 | 5 | 27.094 | 5.4188 | 0.51829 | | |
| ANOVA | | | | | | |
| Source of Variation | SS | df | MS | F | P-value | F crit |
| Between Groups | 772.7464836 | 1 | 772.7464836 | 4.82186 | 0.04999 | 5.31766 |
| Within Groups | 1282.073141 | 8 | 160.2591426 | | | |
| Total | 2054.819624 | 9 | | | | |

Based on low probability value of 0.0499 ($P < 0.05$), it can be concluded that significant relationship exists between sucrose concentration levels and pH of milk at the 43 hours interval.

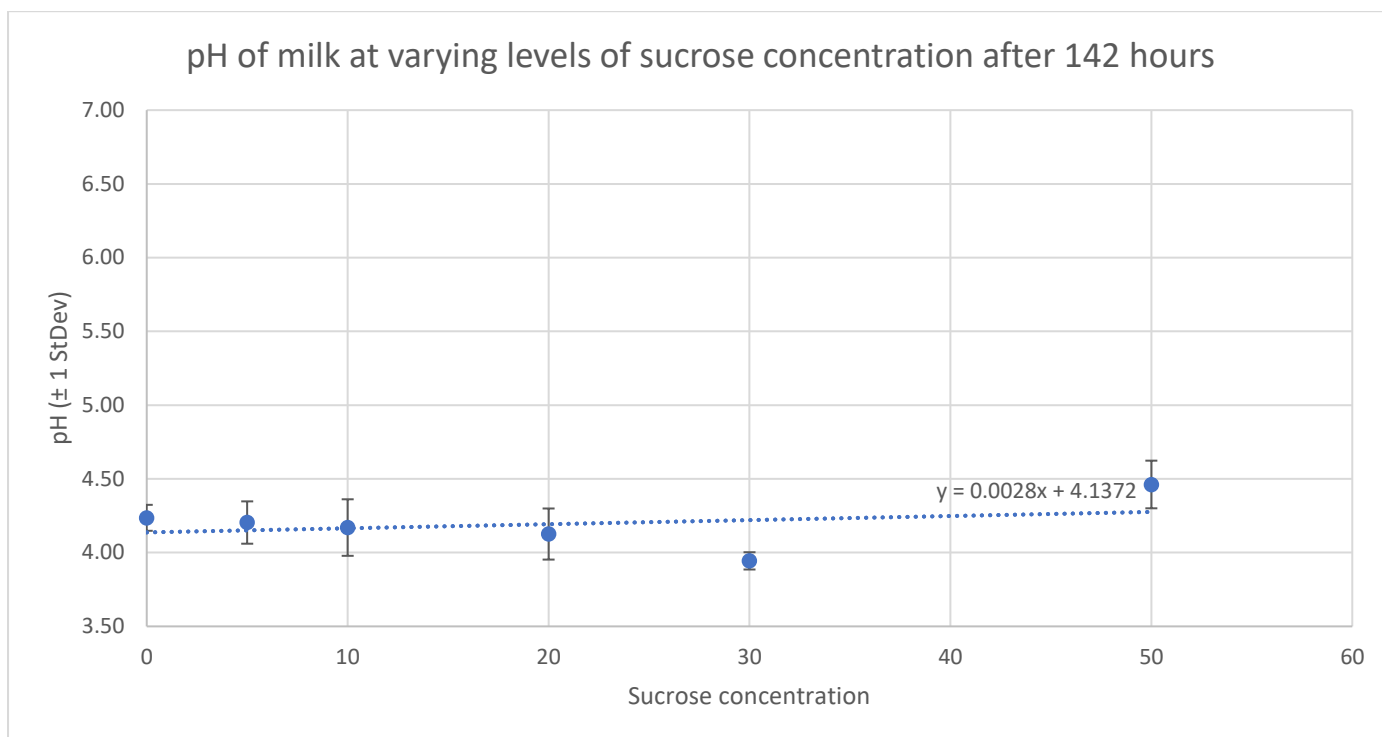
Figure 3: The mean pH of milk sucrose concentration levels at the 73 hours interval with standard deviation error bars



| Anova: Single Factor | | | | | | |
|----------------------|---------|--------|---------|----------|---------|--------|
| SUMMARY | | | | | | |
| Groups | Count | Sum | Average | Variance | | |
| Column 1 | 6 | 115 | 19.1667 | 344.167 | | |
| Column 2 | 6 | 26.892 | 4.482 | 0.39711 | | |
| ANOVA | | | | | | |
| Source of Variation | SS | df | MS | F | P-value | F crit |
| Between Groups | 646.918 | 1 | 646.918 | 3.755 | 0.08138 | 4.9646 |
| Within Groups | 1722.82 | 10 | 172.282 | | | |
| Total | 2369.74 | 11 | | | | |

Based on low probability value of 0.0813 ($P > 0.05$), it cannot be concluded that significant relationship exists between sucrose concentration levels and pH of milk at the 73 hours interval.

Figure 4: The mean pH of milk sucrose concentration levels at the 142 hours interval with standard deviation error bars



| Anova: Single Factor | | | | | | |
|----------------------|---------|--------|---------|----------|---------|--------|
| SUMMARY | | | | | | |
| Groups | Count | Sum | Average | Variance | | |
| Column 1 | 6 | 115 | 19.1667 | 344.167 | | |
| Column 2 | 6 | 25.142 | 4.19033 | 0.02826 | | |
| ANOVA | | | | | | |
| Source of Variation | SS | df | MS | F | P-value | F crit |
| Between Groups | 672.872 | 1 | 672.872 | 3.90983 | 0.07621 | 4.9646 |
| Within Groups | 1720.97 | 10 | 172.097 | | | |
| Total | 2393.85 | 11 | | | | |

Based on low probability value of 0.0762 ($P > 0.05$), it cannot be concluded that significant relationship exists between sucrose concentration levels and pH of milk at the 142 hours interval.

Analysis:

As observed in Figure 1, the pH of milk at all sucrose concentration began to decrease by 27 hours. The pH of milk at sucrose concentration levels 0%, 5%, and 10% reduced the fastest, reaching mean pH of 4.62, 4.69, and 4.40 by 43 hours. The pH of milk at sucrose concentration 20% and 30% decreased at a similar rate overtime, reaching mean pH 4.28 and 4.26 at 73-hour interval. The 50% sucrose concentration milk decreased the slowest over time compared to other sucrose concentrations as it reached the trough of pH by 142 hours, with pH value 4.46. It took milk containing 20% and 30% sucrose concentrations 73 hours to decrease pH around 4.27. This is a longer time compared to 43 hours, which is the amount of time it took for milk at sucrose level 0%, 5%, and 10% to decrease to similar pH value. Similarly, milk with highest sucrose concentration level 50% took 142 hours to decrease to a pH value under 4.50. This implies a negative correlation between increase sucrose concentration in milk and the acidification of milk, indicating higher sucrose concentration levels in milk reduces the rate milk acidifies. As acidification of milk is due to the production of lactic acid, this implies that high sucrose concentration possesses inhibitory property against the growth of *Lactobacillus*, hence limiting the production of lactic acid.

To analyse the statistical relationship between pH of milk and sucrose concentration level, sucrose levels and pH values are taken at intervals 43 hours, 73 hours, and 142 hours to produce Figures 2, 3, and 4.

As Figure 2 demonstrates a linear graph with positive relationship, this indicates that an increase in sucrose concentration level is directly proportional to higher pH value. The corollary is reinforced by results from ANOVA. One Way ANOVA statistical test was applied in Figure 2, which calculated the p-value to be smaller than 0.05, thus showing that the data is statically significant. The results from ANOVA mean that the relationship between sucrose and milk did not happen due to random chances.

In Figure 3, milk with sucrose concentration level 20% and 30% have dropped to pH values 4.28 and 4.26, which is the same pH value of 0%, 5%, and 10% sucrose concentration levels. Compared with sucrose concentration level 50%, a difference of around 1.50 pH is examined. Observing this, two conclusions are made. Firstly, sucrose concentration levels 20% and 30% have little to no effect on pH at 73 hours. Secondly, sucrose concentration level 50% seems to have prevented the decrease of the pH of milk at 73 hours. The first conclusion is supported by ANOVA test which found results to be insignificant, proving null hypothesis to be true – meaning that increase in sucrose concentration does not slow the rate which pH decreases. Nevertheless, ANOVA does not demonstrate where the insignificance lies. Hence, it is important to observe the error bars which represent standard deviation. The second conclusion is attested by error bars; in figure 3, as all error bars of sucrose concentration overlap except for error bar at 50% sucrose concentration, only 50% level sucrose concentration is statically significant compared to levels 0%, 5%, 10%, 20% and 30%. This demonstrates that while sucrose concentration levels 20% and 30% have prevented milk from acidifying for 43 hours, no inhibitory effect is shown at 73 hours. Alternatively, the 50% sucrose concentration level helps to preserve the milk for 73 hours. This may be why a flatter trendline is produced in Figure 3 in contrast to the trendline in Figure 2, reflecting a rather weak correlation between sucrose level and pH of milk.

Shown in Figure 4, at 142 hours all milk at varying sucrose concentration levels decreased to similar pH, creating a rather flat trendline. The minima, flat curve reveals that all sucrose concentration levels of milk, including milk with 50% sucrose concentration, have soured, in evident of the overlapping error bars to show no significant difference between the sucrose concentration levels. This concludes that by 143 hours, all sucrose levels have become ineffective in preventing the souring of milk.

Conclusion:

In response to the research question which investigates the impact of different sucrose concentration levels on the pH of milk, both quantitative and qualitative observations support alternate hypothesis H_1 , suggesting increase in sucrose concentration reduces the rate which pH decreases overtime. Levels of sucrose concentration 20%, 30% reduced the rate which milk acidified for 43 hours in room temperature, and 50% for 73 hours. Therefore, it is concluded that increased sucrose concentration level did extend milk's expiry date, reinforcing that sugar can be added to milk as a conservative to prolong the shelf life of milk (Rawat, 2015).

As stated in introduction, milk spoilage occurs from the production of lactic acid by *Lactobacillus*. Negative casein proteins found in natural milk are repelled from each other by its negative charge ("Why milk curdles", 2009). The production of lactic acid allows the hydrogen ions from the acid to neutralise negative casein proteins ("Why milk curdles", 2009). This causes ionic bonds between lactic acid and casein proteins to result in precipitation of protein, which leads to the curdling of milk ("Why milk curdles", 2009). Thereby, milk curdling is indicative of milk spoilage. It is observed in qualitative data that by 43 hours since milk is stored in room temperature, curdling is shown in milk sucrose levels below 30%, but not in 30% and 50%. This illustrates that high sucrose may have forestalled the growth of *Lactobacillus* by creating a hypertonic environment to dry the cell of water by osmosis (Sehgal, n.d.), hence helping to extend milk's lifespan.

Evaluation

Strengths of the experiment

One strength of the experiment is the large sample size collected in the testing of independent variable on dependent variable. Five trials were conducted for each sucrose concentration, totalling of twenty samples

to assess the impact of sucrose on pH of milk over time. The large sample size provided greater precision of the data in at each sucrose concentration, allowing connections to be drawn and establishing a clear relation of sucrose level on pH of milk over time.

Another strength of the investigation is the high reliability of results confirmed by low standard deviation. A low standard deviation between each trial was obtained for each sucrose concentration, which implies that each trial varied little from the mean. The standard deviation error bars were, therefore, small, implying significant differences in the data collected.

Table 6: Evaluation of limitation and error of the experiment

| Limitation and error | Type of error | Effect | Improvement |
|--|---------------|--|--|
| Sucrose may not fully dissolve in milk | Systematic | Compared to water, milk has a lower solvency. This indicates that milk does not dissolve sucrose as well as water. Undissolved sucrose would render sucrose concentration levels inconsistent and impact on the assessment of IV on DV. | Heating would facilitate the dissolving of sucrose. Heat results in greater molecular movement, which increases the chances of collision between sucrose and milk molecules, facilitating the breakdown of sucrose. |
| Milk solutions were stored in room temperature 25°C | Systematic | Due to limited time to conduct the experiment, milk solutions were stored in room temperature 25°C to accelerate the speed of spoilage to examine the impact of sucrose on pH of milk. Milk is normally stored in the refrigerator within household, not in room temperature, and hence there may be discrepancies in application of results. | Milk can be put in the refrigerator to replicate household settings. More time can be allocated to extend the duration of experiment to make greater assessment of IV on DV in refrigerator temperature. |
| Beakers used to contain milk were not sterilized | Systematic | Other forms of bacteria present in unsterilized beaker may enter the milk, resulting in contamination and potential competitions with bacteria present in milk. This may cause inaccurate measurement of the impact of IV on DV. | Sterilize beakers in autoclave before use. |
| Uncertainties associated with measuring apparatus | Random | The Pasco pH sensor has an associated uncertainty ± 0.1 pH value. (pH of milk) The electric balance as associated uncertainty ± 0.001 g (sucrose) and 100ml measuring cylinder has an associated uncertainty ± 0.1 ml (milk volume). | More trials can be repeated to obtain a more accurate mean (pH) to reduce imprecision of apparatus |
| Potential contamination | Systematic | While taking pH measurements, sucrose milk solutions are placed on the work bench susceptible to the entry of airborne bacteria. | The experiment should be performed under a Bunsen Burner to prevent the entry of airborne bacteria |
| Other forms of bacteria present in milk to contribute to milk spoilage | Random | Other genus of lactic acid bacteria may also be present within milk and produce lactic acid to cause milk spoilage. These bacteria may have different osmotolerance. Hence, the amount of sugar required to prevent the growth of these lactic acid bacteria is hard to gauge. Thereby it is uncertain how much sugar is needed to prolong the duration of milk. | This limitation is difficult to avoid. The milk medium can be separated into individual genus of bacteria to test the impact of sucrose individual bacterium. However, this is difficult to perform with school apparatus. |

Further investigation

The use of room temperature 25°C was identified as a systematic error in my investigation as room temperature does not replicate the temperature which milk is stored in household. An alternative study is to analysis the impact of sucrose level on the pH of milk stored in 4°C (refrigerator temperature).

Nevertheless, if the storage temperature changes, the duration of the experiment will need to change also as milk is found to have a shelf life of around 5 to 6 days in the refrigerator once it is opened. Hence, an appropriate duration would be 14 days to assess the change in pH after shelf life. Hence, the research question would be “How does different levels of sucrose concentration (0%, 5%, 10%, 20%, 30%, 50%) affect the pH of full-cream milk stored in 4°C over a duration of 14 days?”

In my investigation, the interval between 73 hours and 142 hours was observed to be too long of a period. If the experiment was repeated, the pH of milk would be taken at consecutive hours, such as daily, to produce a clearer relationship between the sucrose concentration and pH of milk.

Additionally, different types of milk can be investigated. Examples of such include lactose free milk and skimmed milk, which are just as commonly consumed as full cream milk. Hence, this propounds the research question: “How does different levels of sucrose concentration (0%, 5%, 10%, 20%, 30%, 50%) affect the pH of lactose-free milk stored in 4°C over a duration of 14 days?”

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