International Journal of Science and Research (IJSR)
ISSN: 2319-7064
ResearchGate Impact Factor (2018): 0.28 | SJIF (2019): 7.583

The Effects of Milk Age on the Titratable Acidity of Raw Milk

Kireyna Aurelia Santoso

[1]Sekolah PelitaHarapan Sentul City, Jl. Babakan Madang, Sentul, KecamatanBabakan Madang, Bogor, Jawa Barat 16810, Indonesia

Abstract: ***Titratable Acidity is the percentage of lactic acid in a sample of fluid. TA is used as an indicator in raw milk grading with a value of 0.14 – 0.17% as a healthy range. Two main factors that affect titratable acidity in raw milk are its protein content and age. However, there has been limited research on the effect of storage duration on milk quality, as such analysis have been conducted either with fresh milk or concurrently with other variables that affect the TA of milk. Thus, this paper evaluates the effects of different milk ages (of 5 continuous two-day intervals) on the acidity of raw milk using the method of titration. Titration of raw milk is done with 0.1 M of Sodium Hydroxide. Results show that milk age increases with a positive correlation in correspondence to titratable acidity, due to the multiplication of lactic acid bacteria (LAB) such that the microbial quality directly impacts the rate of fermentation. As the food industry develops, the problems of food additives and safety have attracted much attention, and the only way to prevent those substances from being added is to ensure the evaluation of product through TA values for its chemical parameters that willdetermine the taste and nutrition of the product, as per recommended or required by the government and the NFDA.***

Keywords: Titratable Acidity, Raw Milk

1. Introduction

1.1 Background Information

Milk is widely produced and consumed worldwide as dairy products. Milk has many health benefits, as it is rich in protein, vitamin D and calcium, which are good for bone health and development. Cow's milk is a source of potassium, which reduces blood pressure and sodium levels, which is shown to reduce risks of cardiovascular diseases (Hypertension Institute of St. Thomas Hospital Tennessee). To ensure that the raw milk used for pasteurized milk and dairy products will give optimal benefits and maintain its economic value, its quality is controlled by testing its measure of titratable acidity. Titratable acidity is the percentage of lactic acid in the milk sample, which measures the total acidity in the solution (sum of natural acidity[1] and developed acidity[2]), different from pH which indicates the strength of the acid condition. In milk processing, titratable acidity is used to grade raw milk at the plant intake to study its keeping quality and to measure the heat stability of milk. High quality raw milk has a steady TA value in the range of 0.14-0.17% (KSU Research Center). Acidity of more than 0.18% lactic acid would result in the curdling during heat processing. If TA values of the raw milk surpass the acceptable value, it will fail to meet the desirable flavor, odor, appearance, or shelf life. Hence, processors in the industry set limits on TA to determine what is acceptable into the processing plant.

The method used is titrating a known volume of milk with standard alkali, the titrant (sodium hydroxide) to the endpoint using an indicator. The TA test measures the amount of alkali required to change the acidity of milk from its initial value to its end point.This is calculated by using the formula:

$$X = \frac{M_{Titrant} \times V_{Titrant} \times equivalent\ \ factor}{V_{Sample}\ \ x\ 1000} \text{ x } 100\% \quad (1)$$

Where X is the titratable acidity of the milk test sample, % Lactic Acid,

$M_{titrant}$ is the morality of sodium hydroxide standard volumetric solution, in mol/L

$V_{titrant}$ is the volume of sodium hydroxide solution used during titration in L,

$Equivalent\ factor$ of lactic acid is 90.

V_{sample} is the volume of the milk sample in the beaker in L.

based on the neutralization reaction expressed as

$$C6H5COOH + NaOH \rightarrow C6H5COONa + HOH \quad (2)$$

The two main factors affecting titratable acidity in raw milk are its protein content and age. The age of milk is the duration of storage of milk in the refrigerator before being processed into its end products. As raw milk increases in age, more bacteria grow (UC Davis). Infection of milk by microorganisms can take place during storage, which is analyzed in this paper.

Growth in the bacterial population in raw milk is detrimental because bacteria undergo fermentation, where a chemical breakdown of sugars occur. Lactic acid bacteria, or LAB, is a group if bacteria that live in its natural habitat of milk and starter cultures in production of dairy products. LAB acidifies milk as it produces carbohydrate metabolism of sugars, resulting in the end product of organic acids. Hermofermentive species of LAB convert sugars (glucose, galactose, fructose, lactose as energy resource) in milk into lactic acid, whereas heterofermentive species convert lactose into a composition of 50% lactic acid, 25% acetic acid and ethyl alcohol, and 25% carbon dioxide (Food Science Departement University of Guelph, Canada). Therefore, the main effect of bacterial growth in milk (such as lactocci, lactobacilli, and leuconostoc) is associated with fast acid production and a rapid pH reduction. LAB reduces sugar concentration while simultaneously fermenting lactose to lactic acid, the indicator of total acidity. In a present study

[1] Natural acidity is due to citrates and phosphates present in the milk and dissolved CO2 in milking process

[2] Developed Acidity is the lactic acid produced by the action of bacteria on lactose in milk.

by the Journal of Applied Food Technology, it is shown that bacteria were found to multiply as the storage time increased, especially after the first two days. During refrigerated storage periods, there is a causal link in the increase of the number and the metabolic activity of bacteria.

1.2. Objective

The objective of this experiment is to explore the effects of different milk ages on the acidity of raw milk using the method of titration. 0 days, 2 days, 4 days, 6 days, and 8 days of storage periods (which represents the average days raw milk are stored for before it is processed, because manufacturing centers are large but few in number) are timed to react with 0.1 M sodium hydroxide solution. The increase or decrease in the acidity of milk after each storage period is calculated using the equation of titratable acidity, with the % of lactic acid as the determinant. Hence, the research question investigated is: "How do different storage durations of raw milk (0 days, 2 days, 4 days, 6 days, and 8 days) affect the milk samples' titratable acidity?"

1.3. Hypothesis

As the duration of storage gets lengthened, the raw milk's total acidity in % will increase correspondingly. This is because over time, lactic acid bacteria in raw milk multiply, and the microbial quality directly has an impact of its rate of fermentation (Journal of Applied Food Technology). LAB produces carbohydrate metabolism of sugars, using it as an initial for a conversion to lactic acid (Food Science Department University of Guelph, Canada). This associates with the fast acid production within span of days (KSU). As time passes, more lactic acid bacteria grow, producing lower concentrations of sugars as they are fermented into higher contents of organic lactic acids, resulting in a higher titratable acidity. Therefore, it is predicted that a positive correlation between milk age and Titratable Acidity will be established, meaning that the raw milk tested after 8 days will have the highest % of lactic acid whereas the raw milk tested initially in Day 0 will have the lowest % of lactic acid.

1.4. Variables

1.4.1. Independent Variable: Storage Durations of Raw Milk

Storage durations of raw milk (day, $\pm$ 1 min):

48-hour intervals during storage period of 0 to 192 h (0 days, 2 days, 4 days, 6 days, 8 days) before titration. This data interval varies from 0 to 192 hours to simulate the usual periods of refrigeration before processing in the factory, which takes 0 - 10 days (0 – 240 hours). This means that the largest possible interval is 48 hours, every two days, which was applied within the 5 data points to ensure that the change in the dependent variable is more apparent. The storage duration or milk age in the fridge will be measured by using a timer with an uncertainty of $\pm$ 1 min. A timer is a specialized type of clock specifically used for measuring time intervals in xx:yy:zz (days:hours:minutes), after which it will notify that the required time has elapsed. It is the most suitable timekeeping tool because others like alarms require a setting of a precise clock time (00-24 hours) which is a limitation because the interval used is 48 hours. The stopwatch is not used because it does not remind when the time interval is over, but it denotes the time elapsed since triggered.

1.4.2. Dependent Variable: Titratable Acidity

Titratable Acidity (% Lactic Acid, $\pm$ 0.01%):

This will be manipulated by titrating the standard alkali, NaOH, to the raw milk sample. The volume of 0.1 M NaOH left in the 25 cm^3burette ($\pm$ 0.05 cm^3) is used to determine the Titratable Acidity using Equation (1). The burette is used because it allows for dispensing larger volumes, and because it is the standard tool in titration that allow careful control of the amount of titrant delivered, for which precision is necessary.

1.4.3. Controlled Variables

Table 1: Controlled Variables

Controlled Variable	Method of Control	Rationale
Temperature of storage (6℃)	The raw milk samples are stored in the refrigerator with the set temperature of 6℃ and ensured by using a thermometer with an uncertainty $\pm$ 0.1 ℃	Storage temperature can increase bacterial counts to over-acidification. It is set at 6℃ because milk factories have found that 6 degrees is the optimal temperature for the shelf-life (Journal of Dairy Science). Since it increases microbial quality at higher temperatures, standardizing the temperature throughout 8 days ensures that acidification is not affected by this confounding variable.
Molarity of NaOH added in burette (0.1 M)	25 ml of 0.1 M NaOH is used in all titration conditions.	Since the molarity of sodium hydroxide directly affects the time and volume used for neutralization, standardizing the molarity ensures that the titration process is unaffected by this confounding variable. 0.1 M NaOH was used to reduce risks regarding safety. It is also unnecessary to obtain a high concentration to neutralize the acid in milk.
Volume of raw milk in Erlenmeyer Flask (25 ml)	A 25 ml graduated cylinder with $\pm$ 0.5 ml uncertainty was used to measure 25 ml of raw milk samples, before they are transferred to the 100 ml Erlenmeyer flask with $\pm$ 10 ml uncertainty.	Since the initial volume of the acid directly affects the time when neutralization endpoint occurs, standardizing the volume ensures that titration process is not affected by this confounding variable. The volume is to be kept at 25 ml so that it has an equal ratio to the base.
Initial nutritional value of raw milk (same amount of protein)	625 ml of bottled raw milk from the same brand and type of unpasteurized milk is obtained to acquire same protein content. The total volume is divided so that there are 25 samples (5 independent variables, 5 trials) with a 25 ml volume.	Different protein contents of milk can increase the buffering capacity of milk that resists change in the acidity content. Since proteins contribute to the increase of titratable acidity, standardizing its protein concentration, hence nutritional value, help ensure that acidification is unaffected by this confounding variable.

The experiment's data will be collected from 5 independent variables, which vary based on the storage durations of the milk samples. For each period of the independent variables, 3 trials will be done to increase accuracy and precision.

2. Procedure

2.1 Equipment and Materials

Equipment
- (5) 150 ml clean, dry containers with air-tight lid
- (1) 100 ml Erlenmeyer flask ($\pm 10\ ml$)
- (1) volumetric funnel
- (1) 25 ml titration burette ($\pm 0.05\ ml$) with stand
- (1) Fridge at 6℃
- (1) Thermometer (± 0.1 ℃)
- (2) 25 ml graduated cylinder ($\pm 0.25\ ml$)
- (5) Timers ($\pm$ 1 min)
- (1) Glass rod
- (1) Small pipette

Materials
- (625 ml) raw milk with the same protein content
- (625 ml) sodium hydroxide 0.1 M NaOH
- Phenolphthalein indicator

2.2. Hazards and Methods of Control

Skin contact: Sodium hydroxide is corrosive. Upon contact with skin, it can cause red rashes, burns, pain, and blisters. Effect of pain occurs after minutes or hours.Avoid direct contact by wearing protective clothing (lab coat and enclosed footwear) and chemical protective gloves. Upon spillage, take contaminated clothing off and blot away excess chemical from skin and clothes. Wash with lukewarm, flowing water.

Eye contact: Sodium hydroxide is also corrosive upon eye contact. It may cause burns with redness, swelling, pain, and blurred vision. If the exposure was severe, it can result in blindness as a permanent damage.Avoid direct contact by using safety goggles. If contaminated, gently blot chemical off the face and flush with lukewarm water while holding the eyes open.

2.3 Method

1) Five containers were labelled: Day 0, Day 2, Day 4, Day 6, and Day 8
2) 125 ml of the same raw unpasteurized milk sample obtained from the same brand and milk factory were added to each container using the 25 ml graduated cylinder.
3) The containers were covered with the air-tight lids immediately. Exposure of sample to the atmosphere should be avoided to minimize absorption of water.
4) Containers are put in a refrigerator with a temperature of 6℃, which should remain constant throughout the experiment.
5) The timer was set for the first 6 hours as Day 0, and every 48-hours later, the milk were tested for up to 8 days (192 hours).

Table 2: Duration Specifications

Timer set	Container that was tested
00:06:00	Day 0
02:06:00	Day 2
04:06:00	Day 4
06:06:00	Day 6
08:06:00	Day 8

Titration:
1) While the containers are kept in storage, the burette setup was rinsed and prepared.
2) A 25 ml graduated cylinder was used to measure 25 ml of 0.1 M NaOH, which was then transferred to the burette using a funnel. When transferring to the burette, the stopcock should be in a closed position (horizontal). The starting volume of the burette should read 0 ml at the lowest point of the meniscus.
3) After the first timer rang for 06:00:00, the sealed container labelled Day 0 was taken out. Using a thermometer, the temperature of the solution was ensured to be at 6℃.
4) 25 ml of the milk sample was obtained using the graduated cylinder before it is poured into a Erlenmeyer flask.
5) 2-3 drops of phenolphthalein indicator were added to the conical flask and stirred with glass rod.
6) The flask was placed in the base of the burette stand.
7) The stopcock was tilted back to the vertical position, and the alkali was slowly added (stopcock was closed and opened continuously) drop by drop, while the conical flask was swirled to mix.
8) The addition of alkali is stopped when the end-point is reached, which is indicated by the first definite change to light pink colour that remains constant for up to 20-30 seconds (Refer to Fig 2)
9) The final volume reading of the burette was recorded.
10) The milk sample tested is disposed and the conical flask is rinsed to eliminate error.
11) Repeat steps 6-15 for 4 other trials.
12) Steps 6 – 16 are repeated using the milk sample from container labelled "Day 2" after the timer for 02:06:00 rang.
13) Steps 6 – 16 are repeated using the milk sample from container labelled "Day 4" after the timer for 04:06:00 rang.
14) Steps 6 – 16 are repeated using the milk sample from container labelled "Day 6" after the timer for 06:06:00 rang.
15) Steps 6 – 16 are repeated using the milk sample from container labelled "Day 8" after the timer for 08:06:00 rang.

3. Data Collection

3.1 Raw Data

Table 3: Titre Volume (Volume Difference) of NAOH from 5 trials after Titration with Milk Samples

Day of Storage (day, $\pm$ 1 min)	Final Volume of NaOH recorded in burette in ml ($\pm$0.05 ml)														
	Trial 1			Trial 2			Trial 3			Trial 4			Trial 5		
	Initial	Final	Diff.	Initial	Final	Diff.	Initial	Final	Diff	Initial	Final	Diff	Initial	Final	Diff
0	0.00	5.75	5.75	5.65	11.60	5.95	11.65	17.50	5.85	5.00	10.75	5.75	10.75	16.60	5.85
2	0.00	13.75	13.75	1.35	15.20	13.85	0.00	13.80	13.80	4.80	18.50	13.70	0.00	13.65	13.65
4	0.00	22.30	22.30	0.00	22.35	22.35	0.00	22.30	22.30	0.10	22.45	22.35	0.00	22.25	22.25
6	0.00	30.65	30.65	0.00	31.00	31.00	0.30	30.80	30.50	0.00	30.75	30.75	0.00	30.70	30.70
8	0.00	38.70	38.70	0.00	38.95	38.95	0.00	38.75	38.75	0.00	38.80	38.80	0.00	38.75	38.75

3.2 Quantitative Data

3.2.1. Sample Calculations for Independent Variable

The uncertainties that were involved in the measure of storage duration derive from the use of a timer, which has an uncertainty of ± 1 minute.

Storage Day 0: $\frac{\pm 1 \text{ minute}}{6\ hours} = \frac{\pm 1 \text{ minute}}{360\ minutes} = 0.00277777 \approx 0.0028$

Storage Day 2: $\frac{\pm 1 \text{ minute}}{52\ hours} = \frac{\pm 1 \text{ minute}}{3240\ minutes} = 0.00030864 \approx 0.0003$

Storage Day 4: $\frac{\pm 1 \text{ minute}}{102\ hours} = \frac{\pm 1 \text{ minute}}{6120\ minutes} = 0.00016339 \approx 0.0002$

Storage Day 6: $\frac{\pm 1 \text{ minute}}{150\ hours} = \frac{\pm 1 \text{ minute}}{9000\ minutes} = 0.00011111 \approx 0.0001$

Storage Day 8: $\frac{\pm 1 \text{ minute}}{198\ hours} = \frac{\pm 1 \text{ minute}}{11880\ minutes} = 0.00008418 \approx 0.0001$

3.2.2. Sample Calculations for Dependent Variable

(a) Average Titre Volume for Storage Day 0:

$\frac{T1+T2+T3+T4+T5}{5} = \frac{5.75+5.95+5.85+5.75+5.85}{5} = \frac{29.15\ ml}{5} =$ 5.83 ml

Percentage Uncertainty: The uncertainties in measuring the Titratable Acidity comprise of the use of the burette and the graduated cylinder. The burette has an uncertainty of ±0.05 ml whereas the Erlenmeyer flask has the uncertainty of ±0.25 ml.

(1) Burette: $\frac{Initial + Final\ Reading\ Uncertainty}{Volume\ Average\ of\ IDV\ 1}$ x 100% = $\frac{0.1\ ml}{5.82\ ml}$ = 0.01718213 x 100% ≈ 1.72%

(2) Graduated Cylinder: $\frac{Final\ Reading\ Uncertainty}{Volume\ of\ Milk}$ x 100% = $\frac{0.25\ ml}{25\ ml}$ x 100% = 0.01 x 100% = 1.00%

(b) Titratable Acidity for Storage Day 0:

$X = \frac{M_{Titrant} \times V_{Titrant} \times equivalent\ factor}{V_{Sample}\ x\ 1000}$ x 100% =

Where X is the titratable acidity of the milk test sample, % Lactic Acid.

$M_{titrant}$ is the molarity of sodium hydroxide standard volumetric solution, in mol/L

$V_{titrant}$ is the volume of sodium hydroxide solution used during titration in L,

$Equivalent\ factor$ of lactic acid is 90.

V_{sample} is the volume of the milk sample in the beaker in L.

(1) Convert $v_{titrant}$ volume to L from mL

Average titre volume used for Day 0's titration: 5.83 ml = 0.00583 L

(2) Convert v_{sample} volume to L from mL

All milk samples used in the Erlenmeyer flask is 25 ml, equivalent to 0.025 L

(3) Input into the equation

$X = \frac{0.1 \times 0.00583 \times 90}{0.025\ x\ 1000}$x100%=0.20988%=0.21% Lactic Acid

(c) Total % Uncertainty: Uncertainty in Measuring Instruments x Value of Titratable Acidity

Total % Uncertainty for Day 0:

(Uncertainty in Burette + Uncertainty in Graduated Cylinder) x 0.0021= (1.72 + 2.00) x 0.0020988 = 0.007807536 ≈ 0.0078%

3.3. Processed Data

Table 4: Titratable Acidity of Raw Milk Samples Corresponding to the 5 Different Storage Durations

Day of Storage (day, $\pm$ 1 min)	Average Titre Volume of NaOH (ml, $\pm$0.05 ml)	Titratable Acidity of Raw Milk Sample (% $Lactic\ Acid$, $\pm$0.01%)
0	5.83	0.21
2	13.75	0.50
4	22.31	0.80
6	30.72	1.11
8	38.79	1.40

Table 5: The Effect of Storage Duration of Raw Milk on Its Titratable Acidity from 5 Trials

Day of Storage (day, $\pm$ 1 min)	Uncertainty of Storage Duration	Titratable Acidity of Raw Milk Sample (% $Lactic\ Acid$, $\pm$0.01%)	Uncertainty of Titratable Acidity
0	0.0028	0.21	0.0078
2	0.0003	0.50	0.0184
4	0.0002	0.80	0.0299
6	0.0001	1.11	0.0411
8	0.0001	1.40	0.0519

3.4. Graphs

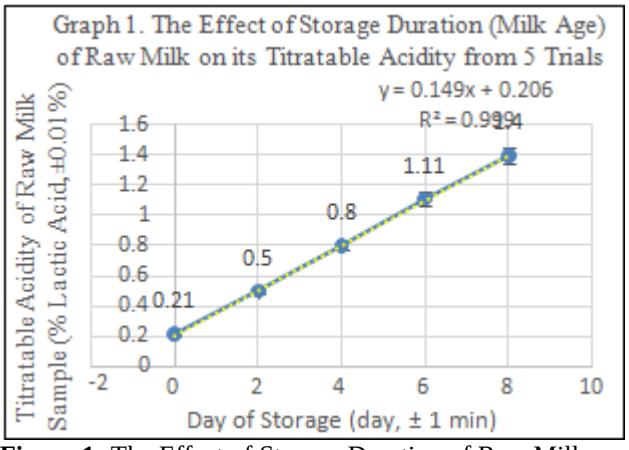

Figure 1: The Effect of Storage Duration of Raw Milk on its Titratable Acidity

3.5 Data Interpretation

The line graph above shows the average of different contents of lactic acid in all five trials after the titration process with the base Sodium Hydroxide. After the standard procedure of storing the raw milk for 6 hours (to disregard the incubation phase) in the refrigerator for the initial titration, the titratable acidity on the first day is found to be 0.21%, and 48 hours later on day 2, it became more acidic with 0.50% TA, 0.80% on day 4, 1.11% on day 6 and 1.40% on day 8.

From the graph, it can be derived from the use of the trendline that the increasing rate of titratable acidity is constantly linear with a difference of 0.29% between day 0 and day 2,0.30% between day 2 and day 4,0.31%between day 4 and 6, and finally 0.29% between day 6 and day 8. The data is progressive and continuous, and the trend shown is a positive correlation.

No significant anomalies or data deviations are indicated in the graph, but the highest increase of total acidity is between day 4 and 6 with a 0.31% rise, and the lowest increase being 0.29% between day 0 and 2, and day 6 and 8. Hence, the trend suggests that the different storage durations of raw milk, having accounted for all controlled variables will affect its titratable acidity. When the storage period is longer by significant amounts of time, the raw milk will have more contents of lactic acid due to the amounts of lactic acid bacterium converting sugars into milk.

It is precise because these data show replicability and reproducibility with very slight variations. This can be identified by how all the data points in the graph cross through the trendline (dotted yellow line), fitting to the rate of increase constantly. The very small range of the error bars indicate the very small variations. The error bars for the horizontal data, indicating the independent variable, is barely visible. This is because of the tool used with a high precision, as discussed later in the validity of method. The uncertainties represented in the error bars through the cap-tipped lines that extend from the centre of the plotted data point, demonstrate the uncertainty of the data point in x and y; a short Error Bar shows that values are concentrated, signalling that the plotted average value is more likely, while a long Error Bar would indicate that the values are more spread out and less reliable. Also depending on the type of data, the length of each pair of Error Bars tend to be of equal length on both sides. However, if the data is skewed, then the lengths on each side would be unbalanced (The Data Visualization Catalogue). This means that the error bars on the graph indicate small variability and very slight uncertainties.

There are five trials for all five variables, which ensure the reliability of the data. All five trials are included for averaging, because none of them are significant outliers (which, in that case, would have been omitted to create better accuracy) and all values obtained are similar. Furthermore, the trend line is used to indicate the general course or tendency of the line that is consecutively linear that describes a pattern of a data with a continuous independent variable that has a proportionate effect on the dependent variable. The set of points functions to see the pattern within the titratable acidity created for each variable. The trend line's progression shows an equation of $y = 0.1495x+0.206$, meaning that for every increase of 2 days or 48 hours in storage duration, there is an increase of y by multiplying the value of x to 0.1495, and adding the answer with 0.206. Furthermore, the R squared value of 0.9999 shows that it is very close to 1.0, which proves the reliability of the data because it means it has a better fit of the regression line (the closer the line passes through all of the points when it is nearer to the value of 1). Any R squared value above 0.8 is considered reliable.

4. Conclusion

4.1 Validity of Hypothesis

Before the experiment, it was predicted that as the duration of storage gets lengthier, the raw milk's total acidity in % will increase correspondingly. Based on research, a positive correlation between milk age and Titratable Acidity was hypothesized, meaning that the raw milk tested after 8 days will have the highest % of lactic acid whereas the raw milk tested initially in Day 0 will have the lowest % of lactic acid. Based on the practical data that has been done, the trend of the graph showing the average of milk age and titratable acidity was also a positive correlation; as the storage duration lengthens, there is a corresponding increase for the percentage of titratable acidity. From graph 1, it can be obtained that the day when the raw milk had the lowest % of TA was on Day 0, which was 0.21%, and the highest % was on Day 2, with the value of 0.50%. This is consistent with the hypothesis. The reason is that lactic acid bacteria in raw milk multiply, and the microbial quality directly has an impact of its rate of fermentation (Journal of Applied Food Technology). LAB produces carbohydrate metabolism of sugars, using it as an initial for a conversion to lactic acid (Food Science Department University of Guelph, Canada). This associates with the fast acid production within span of days (KSU).

Moreover, it is proven by the data labels on the graph that the difference in percentage between day 0-6 keep accumulating by 0.01%. The differences of day 0 and 2, day 2 and 4, day 4 and 6 are 0.29%, 0.30%, and 0.31% respectively. Nevertheless, the rate of increase drops to 0.29% instead of being 0.32% on the next independent variable of day 8. The scientific explanation for this, according to both the National Geographic and the Journal of Dairy Science, when the acidity of the milk reaches a maximum, which varies according to the temperature at which the milk has been held, the acidity will gradually decrease – as lactic acid accumulates, the TA increases to the point where the bacteria can no longer grow, and cease to multiply. Subsequently, the bacteria produce yeasts which grow and reduce the acidity. This research has been proven by Orla Jensen, Rosengren, and others on the bacteriology of butters. The data obtained from this experiment is consistent with other experiments conducted, where titratable acidity in cold storage will never exceed 1.5 percent, the maximum attained in a week. The maximum TA obtained from the experiment is 1.40% on the last day, which is 24 hours above a week's time span.

The strength of the hypothesis is that it satisfies five variables, and the correlation between milk age and titratable acidity was decided upon relevant research from three credible sources. A preliminary experiment for one trial and one variable was also done to ensure that the result from the titratable acidity formula obtained is consistent within the limits of TA of raw milk between 0.16% to 1.50%. However, the limitations of the hypothesis were that no specific numbers of titratable acidity was stated from other findings of the expected outcomes for each day. The result from existing experiments can support for a theoretical yield. However, it was not applied because very few experimental results have been published on this finding, and the available resources provided specifications under different controlled variables from my experiment's. For example, the KSU established a research that under 3 degrees Celsius, the milk will have a titratable acidity of 0.19% on the first day after the first six hours. This is inapplicable and irrelevant to include in the hypothesis because the temperature used, a confounding variable, is different. Another confounding variable to consider is the brand and type of milk used by the KSU and this experiment, which have a variability in protein content. This makes the specifications inapplicable too. Nevertheless, the hypothesis states that the more the duration of storage, the more the titratable acidity. Through the graph progression trends, the hypothesis saying the statement stated above is proven valid, for the reason that the content of lactic acid followed a linear, direct (non-inverse) relationship to the storage duration it is stored for.

4.2 Validity of Method: Strengths, Limitations & Improvements

The procedure's strength was the maintained accuracy and precision. The steps provided specific steps to manipulate the independent variable and its measuring apparatus, a timer, with an uncertainty with the smallest precision of the minute mark, with an uncertainty of $\pm 1\ min$. A timer with indications for days, hours, and minute is appropriate because the seconds and milliseconds are insignificant, while observing the time in minutes is necessary because of the large variability in time it can cause that affects the growth of bacteria in raw milk. Furthermore, a digital timer was used, which eliminates the possibility of human error in the usage of analogue timers. The dependent variable was controlled by the volume left in a burette, of which has an uncertainty of ± 0.05 ml. To ensure precision, the reading of the burette was taken from the meniscus, and the burette was rinsed after each trial. The precision of other tools, for example the Erlenmeyer flask, was also suited with the most precise apparatus available with the volume of 100 ml and the uncertainty of ± 10 ml. Moreover, the second strength is that the materials and equipment are of suitable size and shape (except for the size and uncertainty of the Erlenmayer flask). Each tool and equipment used is used for serving their own purposes in this titration experiment. For example, the Erlenmayer flask is used instead of a beaker because its shape allows for ideal swirling without spills and splashes. The third major strength is the manipulated control of all controlled variables; the environmental conditions were kept constant by storing the raw milk inside a glass air-tight container inside a fridge of temperature 6 degrees Celsius, the NaOH molarity used for all five variables is 0.1 M, the volume of raw milk in the Erlenmeyer flask was always 25 ml, and the nutritional value of the milk was kept the same as 1 L of milk was obtained from the same cow in a milk factory, which means the protein content, another confounding variable, is eliminated. This is a strength because these variables are kept constant and therefore does not become an independent variable, nor does it interfere with the actual end results. All in all, as reflected by the accuracy of the results, the fourth strength is that five trials and five variables were constructed, sufficient amounts for a continuous data to ensure reliability. Since all five trials in each variable state similar values compared to one another, this ensures the data of the accuracy of measurements taken, which is also evident in the trendline from graph 1, that there is no significant anomaly whatsoever. Furthermore, the accuracy of the results is further proven by the very low amount of standard deviation and r-squared value, which means that the method is reliable enough to the extent that replicability of trials will still result in very similar, if not the same, amounts.

In the experiment, the first limitation is a subjective observation of the colour quality, which is both a systematic and random error. Since the methodology of the experiment has the limitation of an unclear end point, observers are not able to completely record when the solution reaches the equivalence point, since the measured pH level drops very quickly (Carpenter, March 2018). This makes determining the exact precise equivalence point difficult, since the colour changes by the indicators, do not change instantly (colby.edu). Moreover, individuals might perceive colour slightly differently, affecting the outcome and results of the experiment. This particular limitation impacts the results because the recorded results might not be the exact equivalence point where neutralization of acid and base occurs, due to the reasons stated above. It affects the data primarily on precision, causing positive deviations from the average value of the measurement. Precision is limited by the random error in regards to the end point, which will affect the whole data range. This titration error shows that the visual end point is always slightly beyond the equivalence point because of the necessity of seeing the color change by eye (Titration Guide: How to Identify and Avoid Titration Errors). The result is that the volume of titrant delivered is too large, giving a larger final concentration than the true value. The measurement estimation error in this circumstance is due to the estimation of the endpoint of the reaction by looking for a specific colour change that can be perceived sensitively. Imprecision could be reduced by averaging over a lot of trials and repeating the measurements, which was done in this case to solve the limitation that shifted the values altogether. The average from the performance of several trials will help errors that fall below the actual value to be accounted for. The more the trials, the more precise the averaged result is. In further experiments, variations of trials could be implemented again and the anomalies could be omitted to maintain precision of the data, instead only using at least three trials that are the closest in measurements. This can be further improved using a "best-fit" line on the graphed data to limit random errors with any slight deviations.

The second limitation, which considers the systematic error and precision is the use of a graduated cylinder to measure the raw milk volume to transfer to the Erlenmayer flask. The graduated cylinder has increments every 5 ml, whereas other tools like the pipette has a higher precision of $\pm 0.03\ ml$. This affects the experiment because a graduated cylinder has a bigger uncertainty; a smaller uncertainty could improve the data accuracy. At first, the use of pipettes were incorporated in the experiment, however it poses challenges since air bubbles are often present, and the raw milk has many curds that makes it difficult to rinse off completely out of the pipette's enclosed structure. In further experiments, some improvements is to use a pipette to measure 25 ml of raw milk. Other improvements are to eliminate air bubbles in the pipette, one should pipette their solutions slowly or pre-wet the tip, and tap the glassware so that the air bubbles pop. To rinse off curds of raw milk and all its contaminants, next time one should clean it with detergent solution or cleaning solution, rinsing it many times with tap water, and repeating the distilled water rinses, and pre-rinsing it before each trial (Purdue.edu). To maintain purity in further experiments, dry pipettes or those that have been cleansed with distilled water and the solution itself should be used, and one should make sure that it has been calibrated. All this could be done in effort of improving accuracy.

The third limitation is the molarity of Sodium Hydroxide used. In this experiment, the molarity of NaOH used is 0.1 M, and this affected the experiment because the volume needed to titrate raw milk samples on day 6 and 8 exceeded the capacity of the burette, so it needed to be measured twice. Another way it affected the experiment is that it takes up a lot of volume of 0.1 M NaOH, hence it takes a longer time to reach the equivalence point. 0.1 M was used initially because no preliminary experiment was done to test out how much base would be needed to balance out the acid in the milk on the last day, so it was not accounted for. Another reason why it was initially used was because it has the lowest risk factor. To make the process more effective, further experiments should consider using higher molarities (preferably 0.4 M or 0.6 M). This improvement would fasten the rate of titration and not waste too much of the basic solution, which is unnecessary as the formula of titratable acidity can be modified to change the molarity of the standard solution (reagent), which will result with a same amount of titratable acidity as when titrated with a lower molarity. Despite some limitations and potential improvements, the procedure of this experiment is valid, because the results do answer the research question and the aim of the experiment, testing the hypothesized concept, with the variable of storage duration influencing the results of titratable acidity.

4.3 Further Extensions

Aside from the potential improvements, an extension that would be beneficial for the betterment of this experiment is to observe the raw milk's titratable acidity for another week, to observe if the trend mentioned above (refer to data interpretation) regarding a deterioration of acidity is valid. Chemists found that once raw milk ages to the point where it is the maximum ability of all four bacteria groups, they will cease to multiply and gradually decrease in rate of acidity, decreasing in TA %. To do this, the same controlled variables should be maintained, but the ranges within the independent variable should be lengthened. Further inquiries are to make investigations based on a different independent variable. Instead of using raw milk age, it would be interesting to find out the effects of protein content (from different cows or different milk factories), or different temperatures of storage. Throughout the research for the experiment, I found that protein content is pivotal in determining the raw milk's acceptability for consumption and production because of its acidity when stored. I also find it interesting that bacteria grow faster in room temperature, resulting in higher acidity, as opposed to colder storage temperatures. This can be manipulated by differentiating three different variables in different storage conditions (fridge temperature of 4-6 degrees Celsius, room temperature of 20-25 degrees Celsius, and blood temperature of 37 degrees Celsius). Something that I would research next time to add to this experiment is a further exploration on the cause of bacterial growth and the limit when it reaches its maximum point, deteriorates, and cease to multiply.

4.4. Implications

The measure of titratable acidity and the factors affecting it is important as it is applied in many real life occurrences. In the economic implications, the use of the TA method is most prevalent in the food industry. The method of TA ensures the economic value of these wines to ensure best customer preference and to ensure compliance with NFDA's restrictions. Total alcohol consumption per capita, based on the amount of alcohol consumption (litres of pure alcohol) per person ages 15+ per year is an average of 8-12 litres globally. Titratable acidity plays an important role as a strength, as it is an indicator for the quantity of organic acids and its salts contained in dairy products and fruits (Journal of Agricultural Sciences). They are used for quality control; for instance, the acceptable titratable acidity of tartaric acid for wine is 4.0-8.0 g/dm^3, and 5.0-8.0 g/dm^3 for must (Wine Folly). Wines with high acidity will be more tart, while low-acidity wines will taste rounder or richer. This benefits the private companies to set up the TAs according to the customers' liking of taste balance, earning them more profit. To control the level of acidity, measures of TA are calculated for further taste modifications. If certain products have less TAs, then there will arise a suspicion on their origin and processing procedure, that some kind of illegal acts were done. Therefore, winemaking and wine stability heavily includes the important role of TA, especially in the sensory of evaluation for finished wines, as acid adjustment and achieving the right acid balance; while the acid can be increased easily by adding citrus juice, it is more difficult to remove it. TA also helps the industry indicate the ability of most bacteria to grow, the solubility of the tartarate salts, the effectiveness of sulphur dioxide, ascorbic acid, enzyme additions, the solubility of proteins, the effectiveness of bentonite, the polymerization of the colour pigments, and oxidative and browning reactions, which determines the wine quality and price (Food Science Toolbox). Furthermore, in wine, those with higher acidic content have a strength as a preservative, slowing oxidation and decelerating the flavour-changing reactions; this quality in wine enhances the economic value in price. The strength of

applying the chemical method of titration to the evaluation of quality is that the process is simple and quick, meaning that it does not have to be controlled by a person with a degree of certain expertise, as long as the person knows the procedure. It is also effective and cost-efficient since it only requires standard lab apparatus. Instead of being time and energy-effective, it is cost-effective for the private sectors and it ensures good quality for the consumers. Although a better representation of perceived acidity than pH, titratable acidity might have some limitations on accurately measuring perceived acidity in beer. The only concerns are whether diluted acids will give accurate results of TA, whether the calculations allow for volumes larger than a 10 ml sample (the standard one), and that it requires large volumes of the titrant 0.1 M NaOH (JBC).

As for dairy products, its economic contribution scopes a large population too; people eat dairy products in all countries of the world, and up to one billion people live on dairy farms. A strength is titratable acidity has been used for many years to indicate whether milk has undergone bacterial degradation (acid production) or temperature abuse or is aged. In milk processing, TA is used to grade raw milk at the plant intake to study the heat stability of milk. If TA values of the raw milk surpass the acceptable value, it will fail to meet the desirable flavour, odour, appearance, or shelf life. Therefore, processors in the industry set limits on TA to determine what is acceptable into the processing plant, but it is not yet established as a criteria of quality. The initial TA of raw milk does not only concern the production of pasteurized milk, but also butter and butter oil, skim milk powder, whole milk powder, condensed milk, cheese, etc. that are important for a food/beverage source for the country and for the trade. The strengths of TA methods is that they are applicable in all of these dairy products. As the New Prairie Press Organization states, the value of TA as an indicator of raw milk quality has been challenged recently, because milk is refrigerated within minutes after it leaves the cow until it reaches the consumer. Raw milk quality is an important issue to both dairy farmers and processors, because it affects the end product use, and hence, their economic value. The limitations to the economic implications, however, is that currently, raw milk quality is only determined by fat, protein, total solids content, bacterial counts, and somatic cell count (U.S. Department of Health and Human Services). Titratable acidity is not one of the pay factors listed on the milk check but has a strong economic impact, because it is one of the criteria used to determine whether or not raw milk enters the food chain as a premium-priced fluid product (New Prairie Press Organization). This is an unfortunate limitation since TA increased as milk protein increases, as bacterial populations and age increases to surpass an acceptable level of 0.17%. However, TA is actually a valid method of evaluating raw milk quality (Kansas University). As for the mechanism of the process itself, there are no economic limitations for milk products. Several private sectors use analytical acid-base reagents that quickly convert a mixture of strong and weak acids into a new system. This conversion makes it possible to directly get the analytical signal (pH, optical density) for titratable acidity calculation. Limitations to this, however, is that it requires a technological program that is not as cost-effective, and the control of stationary conditions should be under close scrutiny. Although the economic factor of this method to obtain TA is more expensive, it does give more reliable results for decrease labour consumption and analysis time, as well as the simplicity of automation (MTF).

Thus, as the food industry develops, the problems of food additives and safety have attracted much attention, and the only way to prevent those substances from being added is to ensure the evaluation of product through TA values for its chemical parameters that will determine the taste and nutrition of the product, as per recommended or required by the government and the NFDA. Effects of consuming too much acid-producing foods and beverages can negative health effects; it may cause a type of kidney stone called uric acid stones to form, bone and muscle deterioration, increasing risks for cancer, liver problems, and heart disease (Healthline). However, the most common concern is that products with high TAs are established with increased titratable acidity in our urine. The strength of titratable acidity and the factors affecting it contributes mainly to the social factor, concerning the issues of health and welfare. When dealing with urine, it is more physiologically meaningful to consider titratable acidity as the amount of alkali required to adjust the pH level of urine back to the pH level of the original blood plasma, which is ~7.4 in all mammalian species (Biochemistry and Molecular Biology Education Journals). In normal urine, the weak acids consist mainly of phosphate and traces of sulfate, as well as small quantities of organic acids, mostly due to the presence of acid phosphates and others like urates, oxalates, pyruvic, lactic, and citric acids. After metabolism, these acids are exerted in the urine as salts of sodium, potassium, calcium, and ammonium (Khata K). Strengths of the usage of TA for testing urine is to indicate a person's immune system health. Many diseases, dietary patterns, and medications taken can affect how acidic or basic a person's urine is, which can be used to establish the likelihood that one's body will form kidney stones. A study of 183 students of the 17-22 age group were studied in respect of fluid intake, urine output, and urine TA under normal conditions for two days (NCBI), and the common average range of TA is 25-35 meq. Since the TA of urine represents the quantity of acid eliminated through the kidneys, the advantage of the TA method is that it can conclude if one's urine is at an extreme on either the low or high values outside of the normal, and thus change lifestyle habits such as the diet and intensity of exercise to reduce the likelihood of painful kidney stones, which can cause pain as they prevent urine from passing through the urinary system. If a person's urine has a TA that is too acidic, they should stop eating/reduce diets rich in acid forming foods, fasting (that produces acetoacetic acid and butyric acids), and vigorous exercise, which all cause the escape of sulphates and phosphates in urine. On the contrary, if a person has an alkaline TA lower than the normal, they should decrease the acidity of urine by a vegetable diet and alkalosis. Since these stones tend to form due to a highly acidic or a highly basic environment, the urine test will be used to determine any health risks. In short, the urine TA is an indicator of overall health, and it gives doctors the idea of what the body is experiencing (Healthline). Another strength is that it determines the best medication to prescribe once a person has a urinary tract infection.

The limitation to this is that studies from the US National Library of Medicine and National Institutes of Health demonstrated that the existing medical and clinical procedures for measuring titratable acidity of urine is not reliable and may result in overestimates of up to 25%. This is a weakness because it can lead to wrong diagnoses and hence wrong medicinal prescriptions. This could lead to unnecessary approaches to either increase or decrease acidity, which might not be the case at all. If handled in such a manner, the issue may lead to a basic or acidic deficit in the body. The Center for Mineral Metabolism and Clinical Research in the University of Texas Southwestern Medical Centre, Dallas, found that the accuracy of the measure is lessened by the loss of CO2, the presence of uric acid crystals and the precipitation of calcium phosphate phases during the titration. To overcome this, hospitals should consider using the new method presented for calculating titratable acidity, by "using a number of routinely-measured urine components and a computer program for calculating complex equilibria in the urine. The results are compiled in a nomogram from which the TA can be directly read."

3.1 References

Number citations consecutively in square brackets [1]. The sentence punctuation follows the brackets [2]. Multiple references [2], [3] are each numbered with separate brackets [1]–[3]. Please note that the references at the end of this document are in the preferred referencing style.

References

[1] "Acid-Alkali Titrations." *Bitesize - GCSE Chemistry*, BBC, www.bbc.com/bitesize/guides/zg34tyc/revision/4.
[2] "Determination Of Activity (Titrable Acidity) Of Milk." *Dairy Science*, Practicals on Technology of Milk & Milk Products Agriculture Information, 2 May 2017, agriinfo.in/determination-of-activity-titrable-acidity-of-milk-702/.
[3] "Lesson 20: Acidity of Milk." *Physical Chemistry of Milk*, UG, 8 Nov. 2012, ecoursesonline.iasri.res.in/mod/page/view.php?id=420
[4] "Sodium Hydroxide." *Canadian Center for Occupational Health and Safety*, OSH, 3 Jan. 2017, www.ccohs.ca/oshanswers/chemicals/chem_profiles/sodium_hydroxide.html.
[5] "Titrable Acidity of Milk." *Dairy Knowledge Portal*, 8 Feb. 2019, www.dairyknowledge.in/content/titrable-acidity-milk.
[6] Bruhn, John C. "A Summary of Titratable Acidity." *Dairy Research and Information Center*, UC Davis, drinc.ucdavis.edu/dairy-food-sciences/summary-titratable-acidity. *Clarification of orange juice by crude fungal pectinase from citrus peel.*
[7] Determination of Titratable Acidity." *Food Science Toolbox*, 17 Feb. 2019, cwsimons.com/determination-of-titratable-acidity-yogurt-samples/.
[8] Rupp, Rebecca. "When Good Milk Goes Bad." *National Geographic*, 8 Sept. 2016, www.nationalgeographic.com/people-and-culture/food/the-plate/2016/09/when-good-milk-goes-bad/.
[9] "Understanding Acidity in Wine." *Wine Folly*, 19 May 2018, winefolly.com/review/understanding-acidity-in-wine/.
[10] "Acidic Foods: What to Limit or Avoid." *Healthline*, Healthline Media, www.healthline.com/health/acid-foods-to-avoid#effects.
[11] Ryan, J. Philip, and Jane A. Irwin. "Practical Studies on Urine Demonstrating Principles of Clinical and Veterinary Significance." *Biochemistry and Molecular Biology Education*, John Wiley & Sons, Ltd, 3 Nov. 2006, iubmb.onlinelibrary.wiley.com/doi/full/10.1002/bmb.2002.494030020019.
[12] Singh, S P, et al. "Study of the Intake of Fluid, Output and Titratable Acidity of Urine in Medical Students." *Indian Journal of Physiology and Pharmacology*, U.S. National Library of Medicine, 1977, www.ncbi.nlm.nih.gov/pubmed/614288.
[13] "What Happens to Wine as It Ages? - Wine Education with Scott Richardson, Retail & Events Coordinator - Wine Education." *Tyrrell's Wines*, www.tyrrells.com.au/education/what-happens-to-wine-as-it-ages-wine-education-with-scott-richardson-retail-events-coordinator/.

Author Profile

Kireyna Aurelia Santoso has won research papers in Indonesian national scientific research paper competitions (LKTIN) and achieved public speaking awards in the national and international scale. She has contributed papers for international conferences on social sciences and has been active in conducting innovative research.