Investigation on Rate of Hydrolytic Rancidity

Research Question:

Comparing the shelf life of skim milk, low-fat milk, and whole milk, to analyze the effect of different amounts of lipids on hydrolytic rancidity.

Introduction:

Milk is the ultimate drink. As our first source of nutrition when we are born, milk can be found anywhere and is regarded as full of health benefits. Children, athletes, and senior citizens value milk for its high calcium content and flavor. Like many other foods, however, milk has a very limited shelf life. Everyone dreads taking out milk from the refrigerator and discovering that it has passed its labeled expiration date. Therefore, the purpose of the research is to compare the shelf life of skim milk, low-fat milk, and whole milk, to analyze the effect of different amounts of lipids on the rate of hydrolytic rancidity, which will be measured through change in pH, volume of water segregation, and qualitative data such as aroma and thickness. One main goal is to discover how big of an impact fat has on the shelf life of milk.

Background information:

Milk is an “aqueous suspension consisting of … carbohydrates (sugars), lipids (fats), proteins, and phosphate”(Wiona State University, n.d.). Shelf life is “the length of time a food can be stored under specified conditions before its quality begins to diminish.” (Derry et. al, 2009, p. 339) Rancid milk has a pungent odor and taste (Cornell University, n.d.) caused by the presence of carboxylic acids, aldehydes, keones and peroxides. It will thicken, and the water will often separate from the milk molecules. The pH value of expired milk is typically 4.4. (Queensland Junior Science, n.d.) In order to test the quality of the milk, one can observe qualitatively, test the pH, and measure the volume of water segregation if there is any.

Rancidity is caused by hydrolysis and oxidation. (Derry et. al, 2009, p. 342)

Hydrolytic rancidity is the breaking down (hydrolysis) of a lipid, producing glycerol and its component fatty acids.
In milk, there are lipids with shorter hydrocarbon chains, which produce short chain fatty acids upon hydrolysis. These produce the flavors and smells typical of rancid dairy products.

Hydrolysis in milk is mostly activated by lipoprotein lipases (LPL) enzymes naturally present in the milk or by the presence of bacteria. Heat can also hasten hydrolytic rancidity (Derry et. al, 2009); therefore, placing the milk samples at room temperature provides energy to activate the lipase enzymes.
The shelf life of milk can also be affected by oxidation. Oxidation involves the addition of oxygen across the carbon-carbon double bond of the unsaturated fatty acid. There are two types of oxidation: normal oxidation, a result of exposure to oxygen, and photoinhibition, a result of exposure to light (Derry et al., 2009).

In this experiment, the focus is on hydrolytic rancidity. Therefore, to minimize oxidation of milk samples, it is important to seal them in airtight jars and cover them from light exposure.

**Prediction:**

As the amount of fat in the milk increases, the rate of hydrolytic rancidity will increase proportionally.
The rate of hydrolytic rancidity will be measured by observing the change in pH over time and by observing qualitative changes in the milk.

Quantitatively, the whole milk will have the greatest rate of change in pH, which is expressed as the largest slope. The 50% should have a smaller slope, and the 10% should have the smallest slope.

\[
\text{Rate of Hydrolytic Rancidity} = \frac{\Delta p\text{H}}{\text{time}}
\]

Qualitatively, the whole milk should have the largest volume of water segregation, the thickest texture, and the sourest smell, while the 50% should have less water, thinner texture, and a less sour smell, and the 10% should have the least water, thinnest texture, and the least sour smell. This is because, the more lipids there are, the more triglycerides will be available to be hydrolyzed into short chain fatty acids, causing hydrolytic rancidity to happen faster.

**Method:**

**Safety & ethical considerations:**

There are no safety or ethical concerns.

**Procedure:**

1. Measure, with a 25±(0.2mL) graduated cylinder, 3 samples of 75.0mL of 100% fat milk, 50% fat milk, and 10% fat milk, each. The same volume of milk and brand of product in every sample ensures consistency for precise comparison. For freshest milk, experimentation begins the day the milk becomes available on the market.

2. Pour samples into nine same-sized separate jars with airtight caps. Label the jars according to their fat content.

3. Measure the initial pH (±0.01) of the nine different samples of milk with a pH probe. Record the smell and thickness. Both quantitative and qualitative data are needed to measure the rate of hydrolytic rancidity.

4. Cap the jars tightly so that there is the same oxygen exposure to the nine jars. Record the time.
5. Place the jars at room temperature, and cover them completely with brown paper towels to prevent light exposure, thus limiting the photo-oxidation.

6. For five days, at the same time, record the change in pH and qualitative data. Make sure that each is exposed to the same amount of air and light each time.

7. After a total of 5 days, if the milk content and water separate, pour the separated water into a 150(±7.5mL) beaker. Use a funnel with filter paper to separate the water from the milk solids. Measure the volume of water with a 100(±1mL) graduated cylinder.

8. Graph the rate of pH vs. time change of the three types of milk and compare, while taking into account the quantitative data, to see in which sample the rate of hydrolytic rancidity was the greatest.

This method was chosen because it combines the use of qualitative data, such as smell and thickness, with quantitative data such as pH and the amount of water segregated from the milk content.

**Raw Data and Data Processing:**

**Qualitative:**

In general, on day 1, all samples were fluid and smelled fragrantly fresh. On day 5, all milks thickened and soured, but the whole milk underwent the most significant thickening and souring while the 10% milk underwent the least. The whole milk thickened and soured fastest, by day 2 or 3, while 50% fat milk did so slower, and 10% fat milk slowest. In trials 2 and 3, the rate the samples thickened and soured was lower than that of trial 1.

**Quantitative:**

Table 1: First Trial Change in pH Value over Five Days

<table>
<thead>
<tr>
<th>Fat Content</th>
<th>1 day (±0.01)pH</th>
<th>2 days (±0.01)pH</th>
<th>3 days (±0.01)pH</th>
<th>4 days (±0.01)pH</th>
<th>5 days (±0.01)pH</th>
<th>Trial 1 Average Rate (±0.02pH units/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%</td>
<td>6.98</td>
<td>6.41</td>
<td>4.31</td>
<td>4.22</td>
<td>4.10</td>
<td>0.58</td>
</tr>
<tr>
<td>50%</td>
<td>6.93</td>
<td>6.81</td>
<td>4.47</td>
<td>4.36</td>
<td>4.21</td>
<td>0.54</td>
</tr>
<tr>
<td>10%</td>
<td>6.99</td>
<td>6.84</td>
<td>5.50</td>
<td>5.19</td>
<td>4.60</td>
<td>0.48</td>
</tr>
</tbody>
</table>
Table 2: Second Trial Change in pH Value over Five Days

<table>
<thead>
<tr>
<th>Fat Content</th>
<th>1 day (±0.01)pH</th>
<th>2 days (±0.01)pH</th>
<th>3 days (±0.01)pH</th>
<th>4 days (±0.01)pH</th>
<th>5 days (±0.01)pH</th>
<th>Trial 2 Average Rate (±0.02pH units/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%</td>
<td>7.00</td>
<td>6.88</td>
<td>4.48</td>
<td>4.30</td>
<td>4.15</td>
<td>0.57</td>
</tr>
<tr>
<td>50%</td>
<td>7.05</td>
<td>7.00</td>
<td>5.97</td>
<td>5.12</td>
<td>4.44</td>
<td>0.52</td>
</tr>
<tr>
<td>10%</td>
<td>7.05</td>
<td>7.03</td>
<td>6.93</td>
<td>5.22</td>
<td>4.72</td>
<td>0.46</td>
</tr>
</tbody>
</table>

Table 3: Third Trial Change in pH Value over Five Days

<table>
<thead>
<tr>
<th>Fat Content</th>
<th>1 day (±0.01)pH</th>
<th>2 days (±0.01)pH</th>
<th>3 days (±0.01)pH</th>
<th>4 days (±0.01)pH</th>
<th>5 days (±0.01)pH</th>
<th>Trial 3 Average Rate (±0.02pH units/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%</td>
<td>7.01</td>
<td>6.89</td>
<td>4.55</td>
<td>4.30</td>
<td>4.16</td>
<td>0.57</td>
</tr>
<tr>
<td>50%</td>
<td>7.05</td>
<td>7.00</td>
<td>6.01</td>
<td>5.15</td>
<td>4.41</td>
<td>0.53</td>
</tr>
<tr>
<td>10%</td>
<td>7.06</td>
<td>7.04</td>
<td>7.01</td>
<td>5.27</td>
<td>4.69</td>
<td>0.47</td>
</tr>
</tbody>
</table>

Table 4: Average Change in pH Value over Five Days

<table>
<thead>
<tr>
<th>Fat Content</th>
<th>1 day (±0.01)pH</th>
<th>2 days (±0.01)pH</th>
<th>3 days (±0.01)pH</th>
<th>4 days (±0.01)pH</th>
<th>5 days (±0.01)pH</th>
<th>Overall Average Rate (±0.02pH units/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%</td>
<td>7.00</td>
<td>6.73</td>
<td>4.45</td>
<td>4.27</td>
<td>4.14</td>
<td>0.57</td>
</tr>
<tr>
<td>50%</td>
<td>7.01</td>
<td>6.94</td>
<td>5.48</td>
<td>4.88</td>
<td>4.35</td>
<td>0.53</td>
</tr>
<tr>
<td>10%</td>
<td>7.03</td>
<td>6.97</td>
<td>6.48</td>
<td>5.23</td>
<td>4.67</td>
<td>0.47</td>
</tr>
</tbody>
</table>

Table 5: Volume of Water Segregation from Three Types of Milk

<table>
<thead>
<tr>
<th></th>
<th>Whole milk (±0.1mL)</th>
<th>50% fat milk (±0.1mL)</th>
<th>10% fat milk (±0.1mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial 1</td>
<td>0.0</td>
<td>23.2</td>
<td>36.1</td>
</tr>
<tr>
<td>Trial 2</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Trial 3</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Sample Calculations: \[\text{Average } pH = \frac{(pH1 + pH2 + pH3)}{3}\]

\[= \frac{6.99 \pm 0.01pH + 7.07 \pm 0.01pH + 7.06 \pm 0.01pH}{3 \text{ trials}}\]

\[= 7.04 \pm 0.01pH (3SF)\]
Analysis:

In trial 1, the rates of the change in pH are higher than that of trial 2 and 3, which have similar average rates (Tables 2, 3, and 4). This is because trial 1 was completed first, while the last two trials were done later, concurrently. The climate was colder later, having the effect of decreasing the rate of hydrolytic rancidity since lower temperature decreases the rate of enzyme activity.

There is not a strong association between pH and time (Figure 4), as seen from the mediocre R² values that are less than one. However, an overall generalized relationship can be seen from the linear trend lines: the whole milk has the largest slope, which shows the highest rate of change in pH, while the 10% fat milk has the smallest slope, which shows the
lowest rate. Overall, whole milk’s pH decreased the fastest. Combining this to the qualitative data, it can be generalized that whole milk has the highest rate of hydrolytic rancidity, 50% fat milk has a lower rate, and 10% fat milk has the lowest. This confirms the prediction that as fat content increases, the rate of hydrolytic rancidity increases.

In trial 1, the 10% fat milk has the largest volume of water segregation, 50% fat milk has some, and whole milk has none. This is opposite of the prediction that rancid whole milk should have the largest volume of water segregation. From other data, however, here is strong proof that whole milk has the highest rate of hydrolytic rancidity. This shows that the volume of water segregation does not express the rate of hydrolytic rancidity, which can be explained because removing fat from milk products causes low-fat milks to have a higher percentage of water content.

**Conclusion:**

This research investigated the effect of different amounts of lipids on hydrolytic rancidity on the shelf life of 10% fat milk, 50% fat milk, and whole milk. By measuring the rate of change in pH, as well as qualitative observations, it can be determined that the higher the amount of lipids, the faster the rate of hydrolytic rancidity, and the shorter the shelf life is. The results corroborate with the prediction, but the fact that water will often separate from rancid milk did not happen in every trial (Table 1), and thus it can be determined that water segregation is not an accurate measure of the rate of hydrolytic rancidity. As a result in the increase of heat energy from the room temperature, the lipase enzyme reactions were activated, causing the hydrolysis of the lipids into glycerol and short fatty acids; the increase of short fatty acids caused the milk to become rancid as expressed through the drop in pH, the sour smell, and the change in consistency. The conclusion that milk with more lipids has a shorter shelf life can help us in our everyday life, because it shows that it is important to keep milk, especially with more lipids, in the cold environment of the refrigerator. Milks of different fat content but the same production date have the same “expiration date” on their cartons, but now it has been proved that low-fat milk, especially 10% fat milk, can be kept longer than whole milk. This also has a major health implication that we need to be more careful with high-fat foods going rancid quickly. In addition, another investigation can be made to see whether in other food and drinks, the higher the fat content, the shorter the shelf life holds true.
Evaluation:

The results of this experiment are inaccurate but precise. Although the experiment includes both quantitative and qualitative data, which increase accuracy by taking both concrete data and the experimenter’s vision and sense of smell, there are many sources of error.

To improve the certainty of the claim that whole milk has the highest rate of hydrolytic rancidity, it is necessary to increase to trials of the experiment and to try different brands of milks from different countries. Repeating at least two more trials would minimize the random error to increase precision.

The pH probe was never calibrated, which caused all the pH values to be either lower or higher than the true value. This systematic error made the quantitative data inaccurate because the pH probe should be calibrated daily. The pH values are precise because the uncertainty of ±0.01 is low. Since all the measurements were made with the same pH probe, precision was not affected. In the experiment, the emphasis was on the rate in the change of pH and not in the pH values; therefore, the fact that the pH probes were not calibrated greatly affects the accuracy of the pH values, but does not strongly affect the precision in the overall trend that the rate whole milk’s pH decreases is higher than that of lower-fat milks.

Every day, to measure the pH values and make qualitative observations, opening the jars and exposing light and oxygen to the milk samples was inevitable. This was a systematic error, which decreased accuracy, because limited light and oxygen were important control factors to restrict the amount of photo-oxidation and oxidation. To improve, one should make the time each jar is opened every day the same so that theoretically, the same amount of oxygen and light will enter.

Another systematic error that affected accuracy was the fact that the control of room temperature most likely changed since trials 2 and 3 happened after trial 1. To improve, it would be necessary to complete all trials concurrently.

The result that whole milk has the highest rate of hydrolytic rancidity is believable because although the results are inaccurate, the precision is quite high, which makes the general trend that whole milk has the highest rate of hydrolytic rancidity likely to be true.

(1950 words)
Bibliography:


