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Article

Heat impact to food's shelf life - An example of milk

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SUMMARY

Food spoilage happens when food is not kept in a good storage condition. A common cause of food spoilage is exposure to high temperatures. Heat exposed food will have a shorter shelf life. Qualitatively estimating the shortened shelf life of food could reduce food waste. In this study, we tested the impact of heat on milk shelf life. We estimated the shelf life loss for milk, which is usually stored at 4°C, after high temperature exposure based on the first-order model with Arrhenius equation. Our results showed that an exposure at room temperature (25°C) for 3.2 hours will decrease the shelf life of milk by one day. With the first-order model and results in the study, we wrote a Python program. The program could predict the loss of the shelf life of milk after its exposure to high temperature for a certain period of time.

INTRODUCTION

Food safety is always an important issue to customers. However, food spoilage and deterioration are spontaneous processes. As soon as we harvest food, it begins to decay (1). Since food is a biological material, oxygen in air, light, moisture, and bacteria can cause its spoilage. The spoilage or deterioration is a complex ecological process, involving the biochemical activity of microbial chemical reactions. Exposure of foods to oxygen triggers a chain of chemical reactions involving proteins, pigments, fatty acids, and lipids, producing other compounds with undesirable biochemical properties, including toxicity, as well as undesirable taste, smell, and color. Many of these processes occur via free radical mechanisms and involve chain reactions (2). For milk, the bacterium *Pseudomonas aeruginosa* plays an important role in its spoilage (3). High temperature increases *P. aeruginosa* levels, which further causes high levels of proteolytic activity (3). Lipases and proteases break down lipids and proteins and the by-products of these hydrolysis reactions increase milk acidity, causing it to spoil (3). Therefore, an acidity test is one method to check for spoiled milk (4, 5).

In order to slow food spoilage or deterioration process, food manufacturers always suggest customer to keep foods at appropriate storage conditions. These conditions include controlling temperature, moisture, and exposure to air, light, and other factors. For perishables, temperature is a key factor. Cold storage or chill-chain transportation works by slowing the chemical reactions or stopping bacterial growth (6).

To guarantee food safety and to preserve their chemical, physical, microbiological, and sensorial characteristics manufacturers set a shelf life for most foods at appropriate storage conditions. There are two main methods used for determining the food shelf life (6). One is a direct method which involves storing the product under pre-selected conditions for a period and checking the product at regular intervals to see when it is beyond the defined quality (6). The other is an indirect method which uses accelerated storage and/or predictive modelling to determine shelf life (6). In this way, people can shorten the trial period with deliberately increasing the rate of deterioration. Usually, increasing the storage temperature speeds up the deterioration process. Shelf life under normal storage conditions is estimated using the results from the indirect method.

Although manufacturers set expiration dates for food stored in the proper storage conditions. However, it is not easy to maintain good storage conditions before customers consume their food. For example, customers may not keep foods at 4°C while driving back home after grocery shopping, or people may store perishable items such as milk or eggs in the refrigerator door, where the temperature tends to fluctuate the most as they opened and closed it. They may also set the refrigerator at a temperature other than 4°C. Sometimes, consumers forget to put food, like milk, in the back of the fridge and/or leave it sitting out at room temperature for too long after use. These temperature fluctuations shorten the food's shelf life. Therefore, evaluation of the impact of heat on shelf life is an important question so food can be used appropriately before its real expiration date.

In this study, we use milk as an example to find the effect of heat impact on its shelf life. We designed an experiment to check milk spoilage at various temperatures with different storage time by an acidity test. We found that an exposure at room temperature (25°C) for 3.2 hours will decrease the shelf life of milk by one day. Our results allow consumers to estimate how short the shelf life will be after they expose milk in a high temperature situation. With this consideration, consumers should use milk before its real expiration date to avoid any food spoilage and reduce the amount of food waste.

RESULTS

To find the effect of heat on the milk's expiration date, we stored the milk at different temperatures. We checked the milk at various time points to find whether it was spoiled with

Temperature (°C)	4	25	40	50
Checking time before spoilage (hour)	360	48	12	6
Checking time at spoilage (hour)	480	54	20	8

Table 1: Time for the milk spoilage at various temperature. Milk was stored at various temperature for different times (8 hours to 20 days) to detect its spoilage (n=1).

an acidity test. With this information, we built a relationship between milk spoilage and its storage condition (temperature and time). With a chemical model, we could predict the shortened shelf life by its exposure at high temperature.

The acidity test used baking soda to react with milk, and acidity (which indicates spoilage) results in bubble generation. We conducted the tests at 4°C, 25°C, 40°C, and 50°C, checked the milk at various time intervals to find when it was spoiled, and recorded the checking time points just before and after spoilage (**Table 1**). From our results, we found that the higher the temperature, the shorter the time to milk spoilage. We obtained a linear relationship between ln(*1/t*) and *1/T* by running linear regression, using Microsoft Excel, on observed values of *t* and *T* (**Figure 1**). The results suggested the degradation of milk with time at temperature between 4°C and 50°C fits the Arrhenius equation (equation 1) with a R^2 more than 0.99 (**Figure 1**).

In the figure 1, t1 and t2 represent the time points just before and after milk spoilage respectively. T represents temperature in Kevin. The equations for the two lines are as below

$$\ln\left(\frac{1}{t1}\right) = 23.405 - 8112.7\left(\frac{1}{T}\right) \text{ and } \ln\left(\frac{1}{t2}\right) = 22.367 - 7890.6\left(\frac{1}{T}\right)$$

The activation energy, E_a, was found from the slope of the linear regression (**Table 2**). The activation energy $E_a(1)$ was from the data for a checking time point just before milk spoilage, and the activation energy $E_a(2)$ was from the data for a checking time point just after milk spoilage. This is because

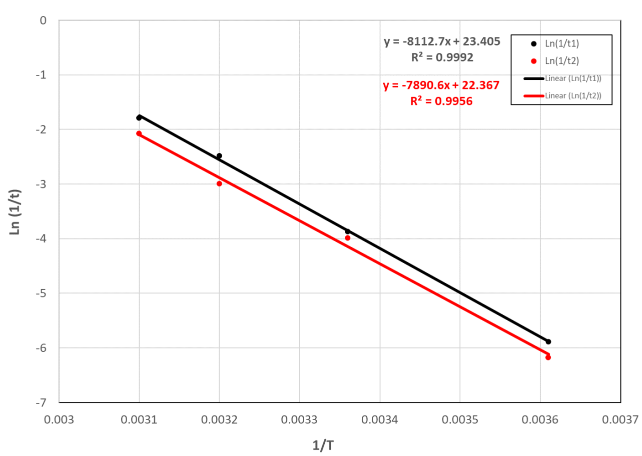

Figure 1: Linear regression curve for *1/T* and Ln(*1/t*). Regressive lines showing a linear relationship between Ln(*1/t*) and *1/T* (*t*1 and *t*2 are times (*t*) when the milk was tested just before and after spoilage, after it was exposed at temperature (*T*) for that long time). Milk was stored at various temperature (4°C, 25°C, 40°C, and 50°C) for different time (8 hours to 20 days) to detect its spoilage (n=1).

P1	*P2*	*Q1*	*Q2*	$E_a(1)$ (kJ/mol)	$E_a(2)$ (kJ/mol)	Ave. E_a (kJ/mol)
23.405	22.367	8112.7	7890.6	67.7	65.8	66.7

Table 2: Parameters for linear regression curves for *1/T* and Ln(*1/t*). Intercept (*P*), slope (*Q*), and activation energy (E_a) of the linear regression lines with a model based on the Arrhenius equation (equation 1) for milk just before (*P*1, *Q*1, and $E_a(1)$) and after (*P*2, *Q*2, and $E_a(2)$) spoilage happened. The slope, *Q*, was equal to $\frac{E_a}{R}$. The activation energy E_a for milk spoilage was calculated as *QR*.

we could not catch an exact time point that the milk just spoiled. Thus, the activation energy for milk spoilage should be in a range between $E_a(1)$ (67.7 kJ/mol) and $E_a(2)$ (65.8 kJ/mol). We took an average of $E_a(1)$ and $E_a(2)$ as the reaction activation energy, which is 66.7 kJ/mol. Then, we calculated an average *P* as 22.89, and an average *Q* as 8001.65 in the same way.

To calculate the shell life of milk at different storage temperatures, we found **equation 7** with substituting P and Q ($t = e^{-22.89+\frac{8001.65}{T}}$). We graphed the relationship between spoilage time (*t*) and temperature (*T*) (**Figure 2**). If we kept milk at room temperature (25°C) for 53 hours, then its shelf life at 4°C would be shortened by 404 hours (17 days) (**Figure 2**).

If we assume milk with only one day's remaining shelf life at 4°C by setting a certain initial concentration C_0, we could find how many hours at a higher temperature it could stay fresh. The P value could be gotten from **equation 8**. $P = \frac{E_a}{2303} - 3.178 = \frac{66700}{2303} - 3.178 = 25.78$. Then, we used this P value into **equation 7**, we can have $t = e^{-25.78+\frac{8001.65}{T}}$ which models another trend between spoilage time and temperature (**Figure 3**). The result showed that an exposure of 25°C for 3.2 hours would decrease the shelf life at 4°C by one day.

DISCUSSION

In our study, high temperature exposure of milk shortened its shelf life, because increasing the temperature of chemical

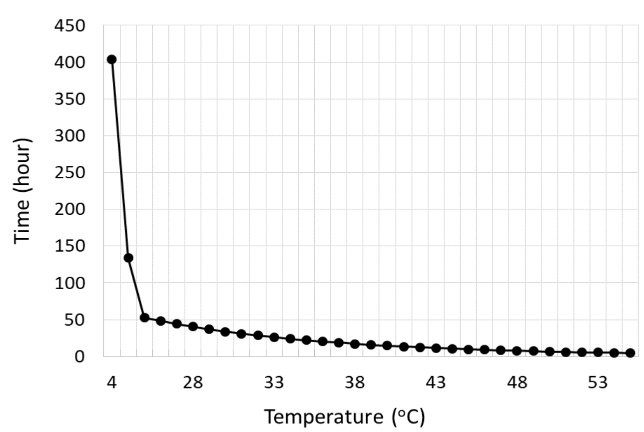

Figure 2: Calculated shelf life of milk based on storage temperature. The predicted time before spoilage for milk with 404-hour shelf life (17 days) at 4°C is exposed at different temperature *T* (°C). An activation energy of 66.7 kJ/mol was used in a model based on the Arrhenius equation (equation 1).

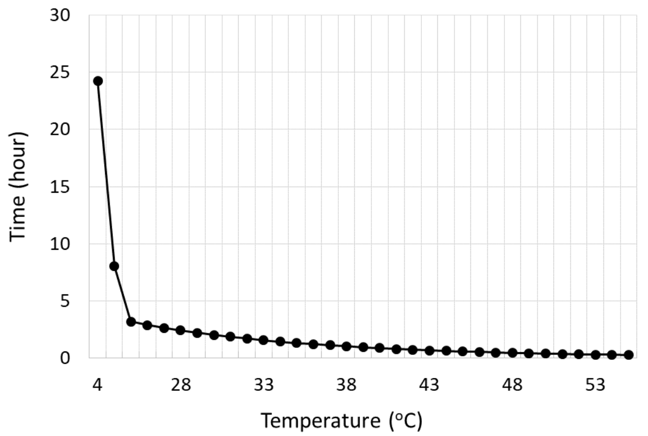

Figure 3: Calculated shelf life of milk based on storage temperature. The predicted time before spoilage for milk with 24-hour shelf life (1 day) at 4°C is exposed at different temperature *T* (°C). An activation energy of 66.7 kJ/mol was used in a model based on the Arrhenius equation (equation 1).

reactions and microbial growth environments accelerates the speed with which these reactions occur. However, there is a limitation for the "high temperature", because at very high temperature, inactivation/damage of microbes and enzymes will happen (7). The inactivation for milk is also called pasteurization, which happens at temperatures over 63°C (8). In pasteurization, heat is applied to foods for destroying pathogens so that the shelf life is lengthened. The International Dairy Foods Association (IDFA) suggests different temperature and duration to perform pasteurization (8). In USA, High Temperature Short Time (HTST) pasteurization is a common process for milk, which uses metal plates and hot water to raise milk temperatures to at least 161°F for not less than 15 seconds, followed by rapid cooling (8).

In real life, the exposure of milk to high temperatures rarely reaches to the pasteurization temperature. In the temperatures we studied, milk's spoilage with time fits the Arrhenius equation (**equation 1**). With the Arrhenius equation, activation energy was derived, and the shelf-life loss was estimated after exposure to higher temperature than the normal storage temperature. This principle could also be used for other foods, like fruit juice, chocolate milk, yogurt, cream, and others. However, for different foods, the activation energy will likely be different. Even for the same brand of milk with different fat concentrations, the activation energies may be different due to the differing fat content. The lower the activation energy, the faster the spoilage process. Thus, a new experiment should be run for each category of food to determine its own degradation parameters to forecast the impact of heat on the expiration date.

With our analysis of the results and the model developed in the study, the heat impact to milk shell life at 4°C can be predicted. Assuming milk is kept at room temperature (25°C) for 53 hours, heat will shorten its shelf life of 17 days (**Figure 2**). Also, one day of shelf life will be lost for every exposure at 25°C for 3.2 hours (**Figure 3**). In real life, container size is likely an important factor affecting the milk's shelf life too. For example, in our experiment, 50 mL of milk was put in a 16 oz glass jar (473 mL), which was immersed in 5 L of water on an oven burner. The heat balance can be reached quickly in this experimental condition. In real life, however, when we put a 1-gallon container of milk (4°C) at room temperature, it takes much longer for all the milk to increase in temperature. The shelf life of the milk after exposure to the elevated temperatures would likely be longer than the one from the current study by using the apparent exposed temperatures. Other factors such as the shape or material of the container could also have other effects on the temperature equilibrium. So, when consumers estimate the shelf life loss, these derivations should be counted in the experiments. With this situation, with simply measuring the equilibrium temperature, the shortened shelf life still could be estimated and give consumers a good reference for the milk's spoilage.

For convenience to get a loss of shelf life of milk after exposure to a higher temperature environment, we wrote a Python program, based on the first-order model with the Arrhenius equation (**equation 1**), to convert its 4°C shelf life to a new one when we exposed the milk to a high temperature (see **Appendix** for code). People could use it to estimate the remaining shelf life of milk to avoid its spoilage before its labeled expiration date due to its heat impact.

In the future studies, the factors of the size, materials and shapes of the containers, and the hot surroundings for the milk should be considered to better fit real life conditions. In this way, a more accurate estimation could be made for the milk consumers.

MATERIALS AND METHODS

Kirkland pasteurized Grade A fat free milk was purchased from COSTCO and kept at room temperature for 15 minutes while driving home. The same milk was used in all tests. 50mL of milk was added into four clean 16 oz glass jars with lids. The glass containers were not re-used to avoid any contamination. One jar was stored at 4°C in the refrigerator as a control, and the other three were sealed and fixed with a weight, and then submerged in water baths with a temperature of 25°C, 40°C, or 50°C, respectively. The water baths were 7.57-L pots (8 quarts) with a cover and were filled with 5 L of water on an oven burner. The oven's power was adjusted to make the water at a constant temperature with ± 2°C. Note that the larger the volume of water, the less the temperature fluctuates.

To determine each sample's degree of spoilage, an acidity examination was conducted at room temperature. 5 g of backing soda was put on a plate, then 2 mL milk was dropped onto the baking soda. The milk was observed for any bubbles to determine how it reacted with the baking soda (4, 5). At the same time, the milk was tasted for its sourness. The testing time points were set. First the time interval was 2 hours, later, it turned 8 hours, or even days depending on the storage temperatures (**Table 3**).

Time	4 °C	25 °C	40 °C	50 °C
0 hours	Test	Test	Test	Test
2 hours	Test	Test	Test	Test
4 hours	Test	Test	Test	Test
6 hours	Test	Test	Test	Test
8 hours	Test	Test	Test	Test
10 hours	Test	Test	Test	
12 hours	Test	Test	Test	
20 hours	Test	Test	Test	
36 hours	Test	Test		
48 hours	Test	Test		
54 hours	Test	Test		
3 days	Test			
4 days	Test			
5 days	Test			
7 days	Test			
10 days	Test			
15 days	Test			
20 days	Test			

Table 3: Time points to test milk's acidity. Test time points for checking milk's spoilage at different temperatures.

Derivation of Equations

As we known, the rate of milk degradation at high temperature depends on its kinetics of the deterioration process. The deterioration process was complex and might involve multiple orders of reactions. Even though, for the kinetics of biomolecular reactions, Eyring and his colleagues developed the most fundamental theoretical model in the 1930s, which leads to an activated complex, known as Transition State Theory (7). Van Boekel gave a more detailed account of its background and derivation in 2008 (9). The empirical equivalent of the Eyring equation is the well-known Arrhenius equation, mostly used in food science (7). The equation relates the rate of product degradation to storage temperature. Historically, the Arrhenius equation has been used to predict real-time product shelf life based on accelerated stability testing at multiple elevated storage temperatures (10). To use the Arrhenius equation, there is an assumption, which asks for no more than a zero or first-order kinetics reaction to take place at each elevated temperature as well as at the normal storage temperature (11). With the products that did not meet the assumption, the food industry developed other model fitting algorithms to describe their non-Arrhenius behavior. Some statistical software packages are commercially available to perform the analysis. With these considerations, we used a first order reaction equation to estimate the loss of shelf life for milk with the test results.

The Arrhenius equation (equation 1) is,

$$k = Ae^{-E_a/RT} \qquad (1)$$

where k was the degradation rate constant; E_a was activation energy; R was gas constant; and T was temperature.

Assuming the initial protein concentration was C_0 and the final protein concentration at the moment of milk spoilage was C_f, then we could express the content at a given temperature in equation 2 as,

$$C_f = C_0 e^{-kt} \qquad (2)$$

where t was time (12).
Replacing k in the Arrhenius equation (equation 1) into equation 2, the new equation 3 became,

$$C_f = C_0 e^{-tAe^{\left(-\frac{E_a}{RT}\right)}} \qquad (3)$$

By dividing C_f and then taking in the natural log of both sides of equation 3, we simplified it into a new equation 4,

$$\frac{1}{t} = \frac{A}{\ln\left(\frac{C_0}{C_f}\right)} e^{-E_a/RT} \qquad (4)$$

Taking in the natural log again of both sides of equation 4 gave us equation 5,

$$\ln\left(\frac{1}{t}\right) = \ln\frac{A}{\ln\left(\frac{C_0}{C_f}\right)} - \frac{E_a}{RT} \qquad (5)$$

By defining $\ln\frac{A}{\ln\left(\frac{C_0}{C_f}\right)}$ as P, and $\frac{E_a}{R}$ as Q, then we could derive equation 5 into equation 6,

$$\ln\left(\frac{1}{t}\right) = P - Q\left(\frac{1}{T}\right) \qquad (6)$$

Here, t was time and T was temperature in Kelvin. Equation 6 builds a relationship between temperature (T) and shelf life (t). With a designed experiment to measure how long the milk can stay fresh at various temperature, we can find slope Q and intercept P. The calculation for t (hr) and T (°C) to Ln (1/t) and 1/T, the plot between the natural log of (1/t) and 1(/T), and the regression line for the plot can be conducted with Microsoft Excel.
Here, $Q = \frac{E_a}{R}$ then, $E_a = Q \times R$. With the slope of the regressive line from the experiments, we can obtain the activation energy. If we rewrote equation 6 into Equation 7.

$$t = e^{-P+\frac{Q}{T}} \qquad (7)$$

Then, we can predict the milk's shelf life at elevated temperature with Equation (7).
In equation 6, Q equals E_a divided by R, which is a constant. However, P equals A divided by $\ln\left(\frac{C_0}{C_f}\right)$ which would vary at different initial concentrations of C_0. If we set C_0 to a value, which had one day's remaining shelf life at 4°C, we could also find how many hours at a higher temperature it could stay fresh. From equation 6, we could derive the value of P at C_0 (one day's remaining shelf life) as

$$P = \ln\left(\frac{1}{T}\right) + Q\left(\frac{1}{T}\right) = \ln\left(\frac{1}{24}\right) + \left(Q \times \frac{1}{273+4}\right) = \frac{Q}{277} - 3.178 = \frac{E_a}{2303} - 3.178$$

$$P = \frac{E_a}{2303} - 3.178 \qquad (8)$$

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APPENDICES

The Python code:

```
import math

def get_new_expiration_date(temperature, time_left_out):
  Q = 8001.65
  temperature_base = 4
    return 1/math.exp(math.log(1/time_left_out)+Q/(273+temperature)-Q/(273+temperature_base))

temperature = float(input("what temperature did you leave the milk at (degrees C)? "))
time_left_out = float(input("for how long did you leave the milk at this temperature (hours)? "))
new_experiation_date = get_new_expiration_date(temperature, time_left_out)
print("your milk has been left out equivalent to " + str(new_experiation_date) + " hours.")
```