Determination of the End of Shelf Life for Milk Using Weibull Hazard Method

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ABSTRACT

The shelf life of pasteurized milk is traditionally estimated by the counts of both total and psychrotrophic microbial load. However, the values reported to date for both microbial populations at the end of the sensory shelf life of milk vary, and are not consistent. The present study examined the relation between the total and psychrotophic microbial growth in milk and its sensory shelf life as measured using the Weibull hazard method. Milk was stored at 5 constant temperatures (2, 5, 7, 12, and 15°C) and both total and psychrotrophic microbial counts were used to obtain the lag time and the growth rate values. The lag time of the total and psychrotrophic growth responded to temperature following the Arrhenius equation. The loss of sensory quality of the milk followed a log shelf life vs. temperature dependency. It was found that there was no correlation between the microbial count at the end of shelf life and the sensory quality of the milk. It is therefore suggested that microbial counts should not be used to determine the sensory shelf life of milk. The Weibull method gave end of shelf life values fairly similar to that of prior work using the ADSA scoring method.

(milk, shelf-life, weibull-hazard)

INTRODUCTION

Undesirable changes in dairy products may be instigated by microbial growth and metabolism or by chemical reactions (17). The determinants of shelf life of fresh dairy products are usually the spoilage bacteria that have the ability to grow at refrigerated temperatures (12, 19). This microbial growth induces changes in the taste and odor of milk such as sour, putrid, bitter, malty, fruity, rancid and unclean (17). In addition, psychrotrophs which are common contaminants in milk, synthesize enzymes, many of which survive the pasteurization heat treatment and during storage biochemically alter the milk and eventually cause spoilage (1, 2).

Growth of psychrotrophic bacteria is predominantly responsible for influencing the keeping quality of milk and dairy products held below 7°C. Raw and pasteurized milk usually spoils when held at refrigeration temperatures because of the effects of psychrotrophic contaminates (3). The populations of microorganisms needed to cause detectable changes in milk varies among genera and species within a genus, but levels at which flavor changes occur are similar at 6 and 20°C (27). Milk spoilage by psychrotrophs was reported in the range of populations of 1×10^2 to 1×10^9 per ml (25). It is therefore unclear whether pschrotrophs counts can be used as an index in the determination of milk quality or shelf life from a sensory standpoint.

As noted earlier, microbial spoilage leads to sensory deterioration of the milk. It may therefore be suggested that the microbial quality of the milk should correlate well to its sensory end of shelf life. The end of shelf life can be determined from sensory data by various graphical methods. The use of hazard rate for shelf life testing of food was introduced by Gacula (1975). Using this method, one can determine the end of shelf life according to the percent of customers a company is prepared to displease (13). The maximum likelihood graphical procedure, or Weibull Hazard method has been used for shelf life of luncheon meats (7), oat bran cereal (21), ice cream (29), cottage cheese (22), Bockwurst sausages and butter (26), and other food products (6).

The objective of this research was to determine whether or not a consumer determined end of sensory shelf life can be described by some microbial index regardless of the temperature conditions that the milk is stored at.

MATERIALS AND METHODS

Milk

The milk used in this study was TLC® fat free milk with added Calcium (Land O' Lakes, Sioux City, South Dakota). This milk is also fortified with nonfat milk proteins. The raw milk was held for no longer than 48 hours at 2°C before processing and then pasteurized (20 s, 80°C). Half gallon, paper board cartons of milk were picked up within 2 h after bottling, taken off the production line consecutively by a plant supervisor, in order to minimize variability between cartons. The cartons of milk were transported to the University of Minnesota on ice in a cooler. Immediately after arrival, the milk was sampled for microbial quality and tasted by three expert dairy panelists to ensure that the milk was of good quality.

Microbial Counts

Total aerobic bacteria as well as psychrotrophic bacteria were enumerated using 3M Petrifilm (3M Co., St. Paul, MN). Samples were diluted in 0.1% peptone, and 1 ml of sample was transfered onto the film in duplicate. The Petrifilm contained standard method nutrients and a cold water soluble gelling agent (8, 20). The bottom film is coated with nutrients and gelling agent, while the top film is coated with the gelling agent and 2,3, 5-triphenylterazoluim chloride (TTC). Colonies appear red and were counted following incubation at 37°C for 48 h for total aerobic or 10 d at 7°C for pyschrotrophs.

Microbial Growth

The growth of total aerobic bacteria and psychrotrophic bacteria in the milk was measured at five constant temperatures: 2, 5, 7, 12, and 15°C (± 1°C). A TempTale (Sensitech, Beverly, MA) temperature recorder, placed in the coolers along with the milk verified the temperature history. Samples were drawn at predetermined intervals

according to the storage temperature conditions. The lag times were determined graphically, and the growth rate constant was calculated through linear regression of the exponential phase of the growth curves.

Sensory Testing

The sensory testing was carried out following the Weibull Hazard method , where the initial number of panelists was $n_0 = 3$ and the constant with which the number of panelists was increased for each subsequent test was $n_c = 1$. The interval between sensory testing was predetermined for each of the five different storage conditions. Because the spoilage of milk at 14°C occurred at an accelerated rate, sensory samples were held overnight so that the sensory test could be carried out at a convenient time for the panelists. The panelists were prescreened and were required to meet the criterion that they consume at least one 8-ounce glass of milk a day. A pool of 33 panelists who met this requirement, 16 male and 17 female, ranging in age form 18 to 45 were available for sensory testing. The panelists were financially compensated according to the number of samples that they tested.

For each sensory test a sample of milk was taken from the milk cartons, poured into a glass flask and the flask was immediately placed into an ice bath to slow down any microbial growth that might have caused any further deterioration of the sensory quality of the milk. Approximately 10 ml of milk was poured into cups that were labeled with random three digit numbers for identification purposes. A tray of milk samples for each of the panelists was prepared approximately half an hour to an hour before sensory testing took place. To ensure that the samples were all the same temperature when the panelists received their trays the trays were stored in conventional home refrigerators held at 4°C. The trays, consisted of samples of milk from the different storage temperatures. The trays were presented to the panelists in sensory booths where the sensory testing was held. Panelists were asked to taste the first sample and determine whether the milk was acceptable or unacceptable, where a response of acceptable implied that the panelist would be willing to drink an entire glass of the

sample. Panelists were asked to wait two minutes between samples and to rinse their mouths with water in between.

The end of sensory shelf life was determined at 69.3 % cumulative hazard or a critical probability of 50%. The data was regressed using the least squares method up to 100% cumulative hazard.

RESULTS AND DISCUSSION

Microbial Growth

The growth of the total aerobic bacteria and the psychrotrophic bacteria were obtained at 2, 5, 7, 12, and 14°C (± 1°C). The lag times and exponential growth rate constants of the total aerobic and psychrotrophic bacteria are presented in Table 1.

Table 1: Growth parameters of aerobic and psychrotrophic bacteria in milk (Lag time – t_L; exponential growth rate constant – k).

	Total Aerobic		Psychrotrophic	
Temperature (^oC)	t_L (h)	k (1/h)	t_L (h)	k (1/h)
14	60	0.094	60	0.104
12	70	0.108	40	0.029
7	225	0.024	225	0.025
5	350	NC	325	NC
2	700	NC	NC	NC

NC – Not calculated.

The total aerobic microbial population exhibited typical growth curves at all temperatures, however, at 2 and 5°C the milk reached the end of sensory shelf life before the end of the lag phase. The psychrotrophic bacteria, exhibit distinct lag and log growth phases at 5, 7, 12, and 14°C. At 2°C however, a rapid growth of

psychrotrophs occurred immediately following the opening of the milk carton for sampling, thus, obtaining a growth curve for psychrotrophs at 2°C was not practical within this experimental setup. It is likely that the rapid growth following opening of the milk carton was due to the change in available oxygen. Once the carton is opened the amount of available oxygen for the microorganisms in the milk increases and facilitates their growth. Sinclair and Stokes (1963) support this explanation with the discovery that in general, due to an increase in the availability of oxygen higher counts are observed.

The growth of both aerobic and pschrotrophs populations at 5°C did not exhibit a distinct logarithmic phase. The absence of a logarithmic growth phase at 5°C can be attributed to the fact that the samples were taken from more than one carton throughout the experiment. Thus the variability in the population of microorganisms from carton to carton may result in difficulties in detecting a distinct lag and exponential phase. The cartons were taken from the production line in consecutive order so that the cartons could be considered to be identical and so the sampling from the cartons during the growth curve study could be made randomly between the opened cartons. However, Maxcy and Wallen (1983) found that heterogeneity between cartons was apparent even when samples were taken sequentially from a single production line.

Growth Parameters

Table 1 shows the duration of the lag phase and the growth rate constants for both types of bacterial counts stored at the five constant temperatures. It was not possible to determine the duration of the lag phase for psychrotrophic bacteria of the milk stored at 2°C because the psychrotrophic counts showed no distinctive pattern. Since the milk stored at 5° and 2°C reached the sensory end point during the lag phase of both the total aerobic bacteria and psychrotrophic bacteria, the exponential growth rates for the bacteria at these two storage temperatures, were not calculated.

Data presented by Fu (1989) showed that the temperature dependence of the growth rate constants and the lag time for microbial growth in a model milk system fits the Arrhenius model:

$$k = k_0 \exp(-E_A / RT) \qquad (1)$$

where k is the microbial growth rate constant, k_0 is the 'collision' or 'frequency' factor, T is the absolute temperature (K), R is the universal gas constant (8.314 J/mol per K) and E_A (J/mol) is the activation energy. The activation energy is a measure of the temperature sensitivity of the reaction(s) responsible for microbial growth. As shown in Figure 1, the dependence of the aerobic and pschrotrophic lag time upon temperature in TLC® milk followed the Arrhenius model.

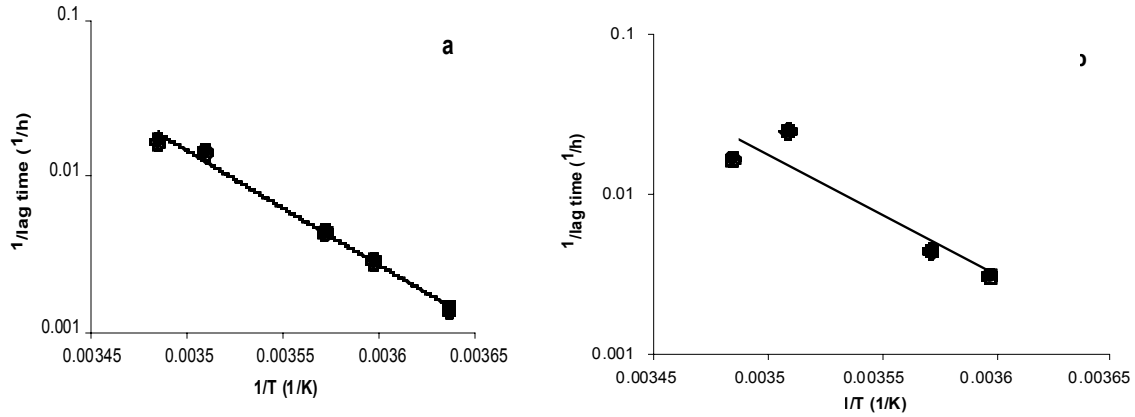

Figure 1: Arrhenius plot of inverse lag times (lag rate) vs. 1/T for the growth of total aerobic bacteria (a) and psychrotrophic bacteria (b) in TLC® milk.

The lag times for the total aerobic counts have a temperature dependence of 14.4 kcal/mol with an $r^2 = 0.99$. The 95% confidence interval of the activation energy is from 12.1 to 16.8 kcal/mol. The fit of the temperature dependence of lag times for psychrotrophic bacteria in TLC® milk to the Arrhenius model had only marginal significance ($r^2 = 0.87$), with an activation energy of 15.5 kcal/mol. However, the 95% confidence interval, -2.2 to 33.2 kcal/mol, is much larger than that for the lag times for total aerobic bacteria. There is one less degree of freedom in this case and this has an impact on the 95% confidence limits. It is therefore suggested that the temperature dependence of the microbial data can be best modeled using the lag times of the total aerobic bacteria.

Sensory Shelf-Life

The end of shelf life at each temperature was determined by the Weibull Hazard statistical method. The end of shelf life was defined as the time that corresponds to a cumulative hazard of 69.3% or a P_C of 50%. The overall results of the sensory test correlated well to the Weibull plot ($r^2 = 0.95$). Figure 2 is an example of a Weibull plot of the sensory results for the milk stored at a constant temperature of 2°C. From the plot, the shelf life in hours is determined as well as the values for the constants α and β. At storage temperature of 2°C, the sensory shelf life of the milk was determined to be 378.5 h and the 95% confidence interval was from 361.9 to 395.8 h. The shape parameter (β), that indicates whether or not the panelists used for the test are biased or not, was 3.3. A β value above 2 indicates that the panelists were not biased (6). Additionally, α, the time when the cumulative hazard is equal to 100% is 423.1 h.

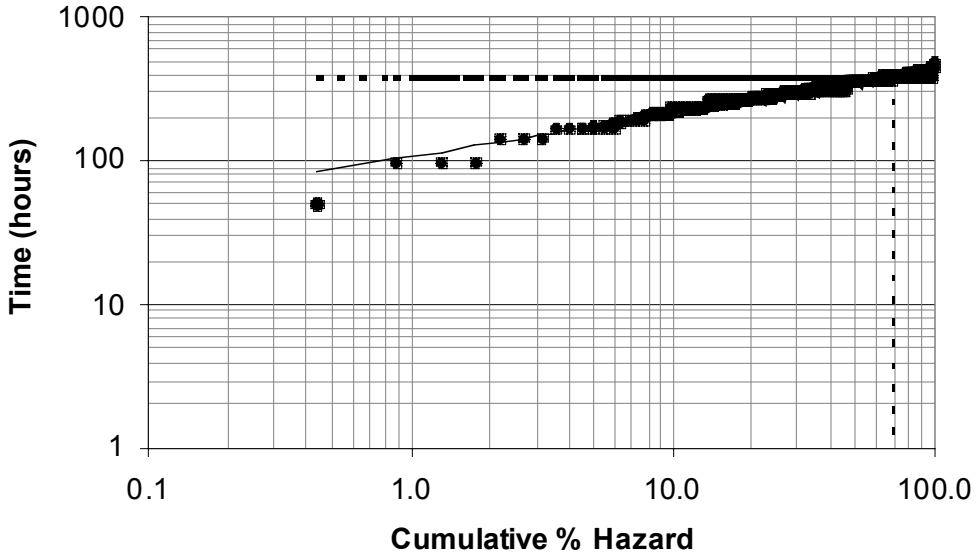

Figure 2: Weibull plot for TLC® milk stored at 2°C.

Table 2 shows the times to the sensory end point of the milk for each of the five constant temperatures as well as the range for their 95% confidence range. The results support the conclusion made by LaGrange and Hammond (1993) that fluid market milk is expected to remain fresh and appealing to customers for 12-14 d when the milk is stored below 4°C.

Sensory quality of milk is commonly evaluated by two dairy experts using the ADSA flavor scoring system where at a score of below 36, the milk is considered as unacceptable. This method was used in a number of studies to evaluate the sensory shelf life of milk as related to temperature (11); temperature and microbial count (4); temperature, code date and microbial count (Hankin et al., 1977). Figure 4 shows that the results obtained in the present study, are in very good agreement with the previous results for sensory shelf life of milk. This demonstrates that the Weibull hazard method correlates well very with the standard ADSA procedure.

Microbial Populations at the Sensory Endpoint

The total aerobic count and the psychrotrophic counts at the sensory end point of the milk are shown in Table 2.

Table 2

Sensory end point, and microbial counts of TLC® milk stored at constant temperatures for a P_c of 50%.

Storage Temp. (°C)	Shelf Life (d/h)	95% confidence interval (h)	Total Aerobic* (cfu/ml)	Psychrotrophic* (cfu/ml)
2	15.8 / 378.5	361.9-395.8	$2x10^2$	$5x10^1$
5	13.7 / 328.1	292.5-368.1	$3x10^2$	$3x10^2$
7	12.3 / 294.6	271.0-320.2	$2x10^4$	$2x10^4$
12	4.6 / 110.9	101.3-121.3	$4x10^7$	$3x10^4$
14	3.9 / 93.2	89.9-96.6	$8x\ 10^4$	$2x\ 10^3$

* estimated at end of shelf life

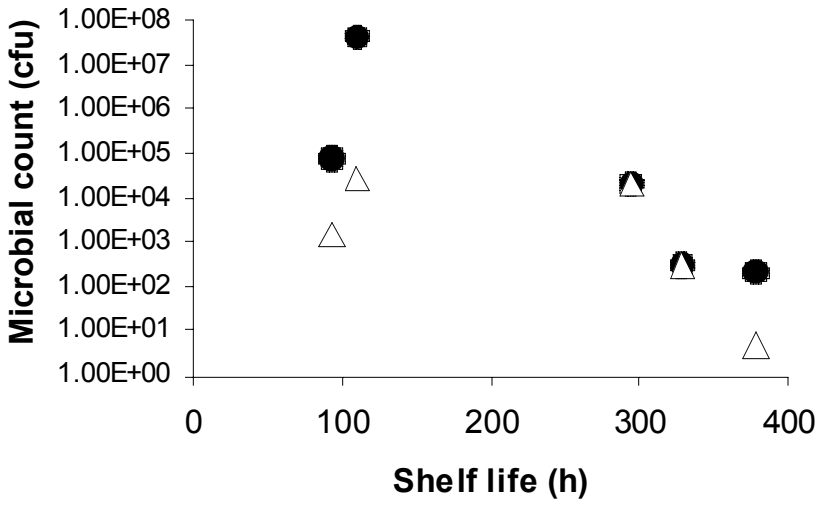

Figure 3: The relation between the total aerobic (●n) and psychrotrophic (Δ) microbial counts and the sensory end of shelf life.

As demonstrated in Figure 3 the correlation between the total aerobic or psychrotrophic count and the sensory shelf life of the milk is poor. Previously, an arbitrary microbial population of 5 x 10^6 cfu/ml (9, 17) has been selected as the index for the end of shelf life for pasteurized whole milk. The present results indicate that the practice of using a microbial population as an indicator for the end of shelf life may not be valid for specialty milk. The failure of total and psychrotroph microbial counts to predict the end of sensory shelf life may be attributed to the fact that the species responsible to sensory retardation are only a part of the population. Since different species have different temperature sensitivities, the storage temperature has an influence on which species pre-dominate (14). Since different microbes produce different off flavors, the nature of the dominant strain type (proteolytic, or lipolytic, etc.) is critical from the standpoint of sensory shelf life. Labuza (1982) points out that to choose an arbitrary microbial load such as 10^6 cfu/ml for the end of shelf life may not be correct and that perhaps a load of only 10^4 cfu/ml of a particular species may result in an off-flavor. The question of total microbial counts as an indicator for milk quality is also important from the regulatory standpoint. The Pasteurized Milk Ordinance (28)

stipulates that milk must have a total aerobic count of less than 2 x10^4 cfu/ml after processing. From the data in Table 2, the milk stored at 7, 14 and 12°C, although legal after pasteurizing, would reach the 20,000 cfu level before the end of the sensory shelf life is reached. If the milk was not open dated, i.e. with no date, an inspector would not know how old it was and could assume it was supposed to be fresh and thus illegal. Only 30 states have open dating laws and their definition of milk shelf life varies from 96 h to 14 d (16). The milk stored at 2 and 5°C does not present a concern since in both cases, at the sensory end point of the milk, the milk has not reached the 20,000 cfu/ml level. It should be noted that at 5 and 2°C a total aerobic count of 2 x10^4 cfu/ml would occur some time after 37.5 d (900 h) of storage. However, the quality of the milk at these storage temperatures was found to be unacceptable after about two weeks so microbial testing of the milk as a quality indicator of the milk is pointless when the milk is properly stored. It thus seems that sensory testing is the method of choice for shelf life dating as was suggested by Maxcy's and Wallen's (1983).

Temperature Dependence of the Spoilage of TLC® Milk

The effect of temperature on the sensory quality loss of TLC® milk was evaluated through a plot of log shelf life vs. temperature as shown in Figure 4 (r^2 = 0.94). As seen, the shelf life data obtained using the ADSA scoring system from the studies of Finley et al. (1968), Janzen et al. (1980), and Hankin et al. (1977) all correlated very well to the temperature (r^2 = 0.91) and to the results using the Weibull method. The temperature dependence of milk sensory deterioration can be expressed as Q_{10} which is the ratio of shelf life of two temperatures 10°C apart on a shelf life plot as demonstrated in Figure 4. The Q_{10} for TLC® milk at 5°C was ~3.6, while it was ~6, ~2.8 and ~2.2 for the results obtained using the ADSA method as reported by Finley et al. (1968), Hankin et al. (1977) and Janzen et al. (1980) respectively. In

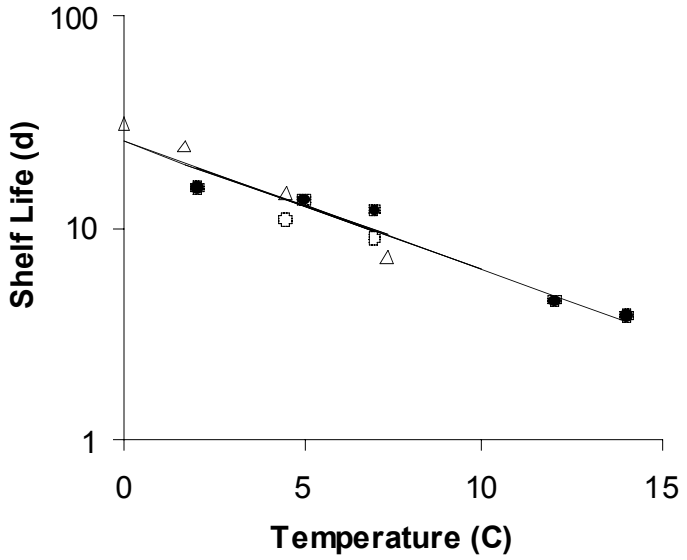

Figure 4: Sensory shelf life plot for pasteurized TLC® milk (●n), as compared to pasteurized whole milk as determined by Hankin et al. (1977) (Δ), Janzen et al. (1980) (❍), and Finley et al. (1968) (■).

those studies the temperatures used were 0, 1.7, 4.5, and 7.3°C for Finley, 1.7, 5.6, and 10°C for Hankin, and 4.5 and 7°C for Janzen. Obviously the differences are due to both the range and the actual temperatures used. Combining all the data gives a Q_{10} of about 4.0, and all the data fit well to a straight line (r^2 = 0.93), thus justifying the Weibull method to the ADSA scoring method.

The activation energy of the sensory retardation of TLC® milk was calculated by using the method perscribed by Taoukis et al. (1997). In the present case for milk the quality function takes the form

$$\ln(Q_f) = \ln\left[\frac{\ln\frac{A_0}{A}}{t_s}\right] = \ln k_0 - \frac{E_a}{RT} \qquad (2)$$

where Q_f is the quality function t_s is the sensory endpoint, T is the temperature in Kelvin, A_0 is the initial percentage of quality (100%), A is the percent of remaining quality at the end of shelf life (50%), R is the universal gas constant (1.987 cal/mol per K) and E_a is the activation energy in kcal/mol. Since the A_0/A value is constant, one can then plot $\log_{10}[1/t_s]$ vs. 1/T °K to get the activation energy as shown in figure 5 using a semi-log plot.

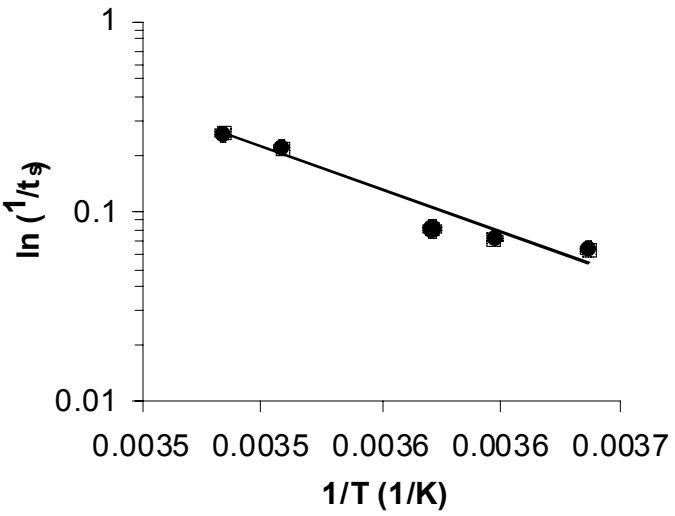

Figure 5: Arrhenius plot of log sensory quality of TLC® vs. 1/T for TLC® milk using zero order quality function.

As seen in Figure 5, the sensory shelf life data fit this equation well ($r^2 = 0.94$), and the activation energy of the quality loss of TLC® milk was calculated to be 20.2 kcal/mol. The range of the 95% confidence interval for this activation energy is from 10.8 to 29.6 kcal/mol. The difference in activation energy between the quality loss (20.2 kcal/mol) and the lag times for the total aerobic bacteria (14.4 kcal/mol) was not significanty different ($P < 0.001$). However, the activation energy for the quality loss was higher than the activation energy for aerobic bacterial growth, thus indicating a higher temperature sensitivity for sensory quality.

CONCLUSIONS

The results of the present study suggest that microbial count may not be a valid tool for determining the sensory shelf life of TLC® milk. It was shown that the sensory quality of milk is more sensitive to temperature than the lag time of the microbial populations, and that the microbial count at the sensory end of shelf life is poorly correlated with the sensory shelf life. These results are in agreement with the work of Yu and Chang (1996) who also demonstrated that the bacterial load in milk is not reliable to determine the quality of a pasteurized fluid whole milk. It is therefore suggested that the best way to determine the sensory endpoint of milk is by sensory testing and not by plate count method.

ACKNOWLEDGMENTS

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