CORRELATION OF TIME-TEMPERATURE INDICATOR RESPONSE WITH MICROBIAL GROWTH IN PASTEURIZED MILK

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ABSTRACT

Commercially obtained pasteurized whole milk was stored at three constant temperatures (0°C, 5°C, and 10°C), and one variable temperature condition (cyclic exposure of 0°C for 14 days and 10°C for 2 days). Daily analyses were conducted to enumerate the growth of total bacteria, coliforms, psychrotrophs, and spore forming organisms in samples from each storage treatment. Microbial growth was correlated with the response of the I-POINT and LifeLine full-history time-temperature indicators. Response of the I-POINT model 2140 was strongly related to germination of the psychrotrophic bacteria, and significant correlations ($r > 0.95$) were found between total count enumeration and the LifeLine model 57 indicator.

INTRODUCTION

Retailers in this country rely on the processor expiration date for quality maintenance of pasteurized milk. An expiration date is a valid quality assurance tool only if the product is held at specific constant temperature conditions during all phases of transport and handling. Pasteurized milk is usually stored between 4° and 10°C, however storage temperatures may be poorly controlled during distribution, and the resulting temperature fluctuations can have tremendous impact on the growth of spoilage bacteria. Much of the uncertainty related to the growth of bacteria in milk could be eliminated by monitoring the cumulative time and temperature exposure which a product receives during distribution.

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Commercially available time-temperature indicators provide a simple means to monitor cumulative time and temperature exposure. Review articles by Scheon and Byrne (1972) and Kramer and Farquhar (1976) provided information on patented and commercially developed indicators that monitor changes in temperature with time. There are two classifications of time-temperature indicators (Wells and Singh 1985). Devices which respond only after a predetermined threshold temperature has been exceeded are said to be "partial-history" indicators, whereas devices which respond to all temperature conditions encountered during storage are called "full-history" indicators. Storage study investigations conducted by Singh *et al.* (1984 and 1986) demonstrated the use of full-history time-temperature indicators as monitors of changes in specific attributes of food quality.

Mistry and Kosikowski (1983) investigated the use of indicators as quality control devices for fluid milk, and concluded that a combination of different models of the I-POINT time-temperature indicators could be used to predict milk spoilage. This work however did not examine the use of time-temperature indicators as monitors of the growth patterns of different microorganisms. The development of an electronic time-temperature indicator based on the growth rates of specific organisms observed in foods has discussed by Ratkowsky *et al.* (1982).

After examination of the Lifeline and I-POINT full-history time-temperature indicators, Wells and Singh (1986) concluded that these two kinds of indicators respond in a reproducible fashion over both extended periods of time, and wide ranges of temperature. Enzymatic or chemical reactions within each of these indicators proceed with time, and are accelerated with increased temperature. The enzymatic or chemical reactions either result in the formation of colored products or cause pH changes detected by a color indicator. In turn, the color changes are monitored either visually, with the aid of a color reference scale, or electronically, with an optical scanner. Thus, an indicator's color directly reflects the temperature history to which the indicator has been exposed.

The objective of this research was to evaluate the performance of two commercially available full-history time-temperature indicators when correlated with the microbial growth in pasteurized whole milk stored at refrigeration temperatures.

MATERIAL AND METHODS

Pasteurized Whole Milk

One pint cartons of homogenized whole milk obtained from the Crystal Cream and Dairy Company (3013 D Street, Sacramento, CA) were used in this in-

vestigation. The milk samples were pasteurized (76 °C for 16.5 s) in the dairy, the evening before the beginning of the study, and were delivered to the university laboratory the following morning. Case lots of 36 milk cartons each, were randomly assigned to one of four treatment groups. Three treatment groups were placed into cold rooms strictly maintained at 0 °, 5 °, and 10 °C for the duration of the study. The fourth treatment consisted of a variable temperature storage cycle of 14 days at 0 °C, followed by 2 days storage at 10 °C. Initial microbial analysis began six hours after samples were placed in cold storage.

Microbial Testing Procedures

Each morning bacteria from two cartons were enumerated. Serial dilutions of milk were made in 0.1% Bactopeptone, and duplicate pour plates were prepared using four different media. Standard Methods Agar, incubated at 30 °C for 48 h, was used to determine total counts. Coliforms were enumerated using Violet Red Bile Agar incubated at 37 °C for 24 h. Psychrotrophic bacteria were detected by plating on Standard Methods Agar incubated at 7 °C for 10 days. Spore counts were obtained by incubating the milk in Dextrose Tryptone Agar for 30 min at 80 °C, plating, and further incubating the media at 30 °C for 48 h. All counts are expressed as colony forming units (CFU) per mL milk.

Time-Temperature Indicators

The I-POINT Time/Temperature Monitor (I-POINT Technologies, Malmo, Sweden) and the LifeLine Inventory Freshness Monitor (LifeLine Technologies, Morristown, NJ) were studied in this investigation. The LifeLine indicator contains an acetylenic monomer that changes color irreversiblely as a result of polymerization. The color change is quantified with a hand-held microcomputer and an optical wand which indicates the decreases in light reflectance (100%-0%) as the indicator darkens. The I-POINT indicator contains a proprietary enzyme and substrate that undergo a hydrolysis reaction. As the hydrolysis reaction proceeds the solution pH changes, which in turn is exhibited as a gradual color change by a pH indicator dye. The color change is visually compared to a reference color scale which corresponds to four discrete increments (0, 1, 2, and 3).

Ten each of the I-POINT Time/Temperature Monitors (model 2140) and the LifeLine Inventory Freshness Monitors (model 57) were attached one per carton to representative milk cartons in each storage treatment (Fig. 1). Indicators were inspected within their respective storage room, at 3 and 4 day intervals. The cartons with indicators attached remained in storage until the conclusion of the study, and were not included in microbial sampling. At the end of the investigation, indicator response was correlated with changes in the selected microbial populations according to the procedure established by Singh *et al.* (1984).

FIG. 1. LIFELINE FRESHNESS MONITOR MODEL 57 (LEFT) AND I-POINT TIME/TEMPERATURE MONITOR MODEL 2140 (RIGHT) PLACED ON 1-PINT CARTONS OF PASTEURIZED MILK

RESULTS AND DISCUSSION

Effect of Temperature on Microbial Growth

Microbial growth profiles obtained at the four different treatment conditions are shown in Fig. 2a, 3a, 4a and 5a. The initial bacterial counts agree with recent reports of other investigators (Credit *et al.* 1972; Langeveld *et al.* 1972; Langeveld and Cuperus 1980). The keeping quality of the milk, as assessed by the time required to reach 5×10^6 CFU/mL (Langeveld and Cuperus 1980), is indicative of a very low level contamination by psychrotrophic gram-negative rods. The CFU/mL exceeded 5×10^6 only after 20 days at 5 °C and after 10 days at 10 °C. In contrast, Schroder *et al* (1982) reported that milk experimentally contaminated with small numbers of gram-negative rods contained more than 2×10^6 CFU/mL in three days at 5 °C and in as little as six days at 11 °C. These psychrotrophs are invariably post-pasteurization contaminants (Langeveld *et al.* 1972), and very few are needed to initiate spoilage (off-flavors) as the most effective competitors are destroyed by pasteurization (Dulshretha and Marth 1975).

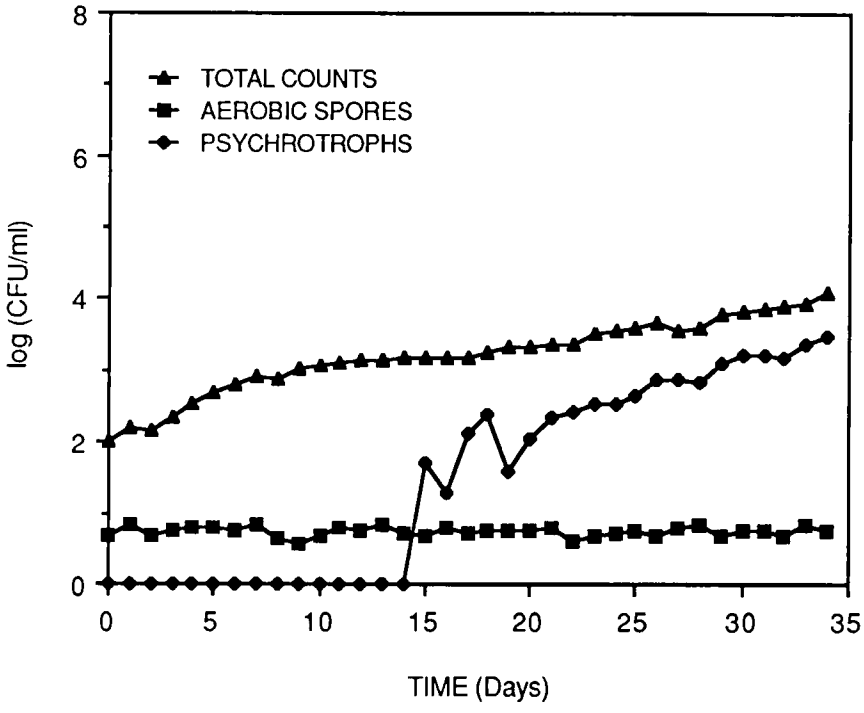

FIG. 2a. CHARACTERIZATION OF THE MICROBIAL GROWTH IN PASTEURIZED MILK FOR THE 0 °C STORAGE TREATMENT

The microbial growth pattern observed in this investigation was more indicative of contamination by *Bacillus*, presumably spores that had survived pasteurization. At refrigeration temperatures, psychrotrophic *Bacillus* species grow much more slowly than do the commonly occurring psychrotrophic pseudomonads (Langeveld and Cuperus 1980). Psychrotrophic Bacilli contaminants are not unusual. Overcast and Atmaram (1974) reported that almost 30% of commercially pasteurized milk samples stored at 7 °C were spoiled by *Bacilli cereus*. Credit *et al.* (1972) reportd that milk experimentally contaminated with *Bacillus* spores which had survived pasteurization showed signs of spoilage only after 30 days at 4.5 °C. In the present study, microscopic examination of bacteria constituting the predominant colony types of the total count population did, in fact, reveal large spore-containing gram-positive rods.

The seemingly contradictory finding that spore counts did not simultaneously increase is probably the result of inefficient sporulation in milk stored at low temperatures (Shehata and Collins 1972; Rodriquez and Barrett 1986). Exposure to a carbon or nitrogen limiting environment stimulates spore production in most

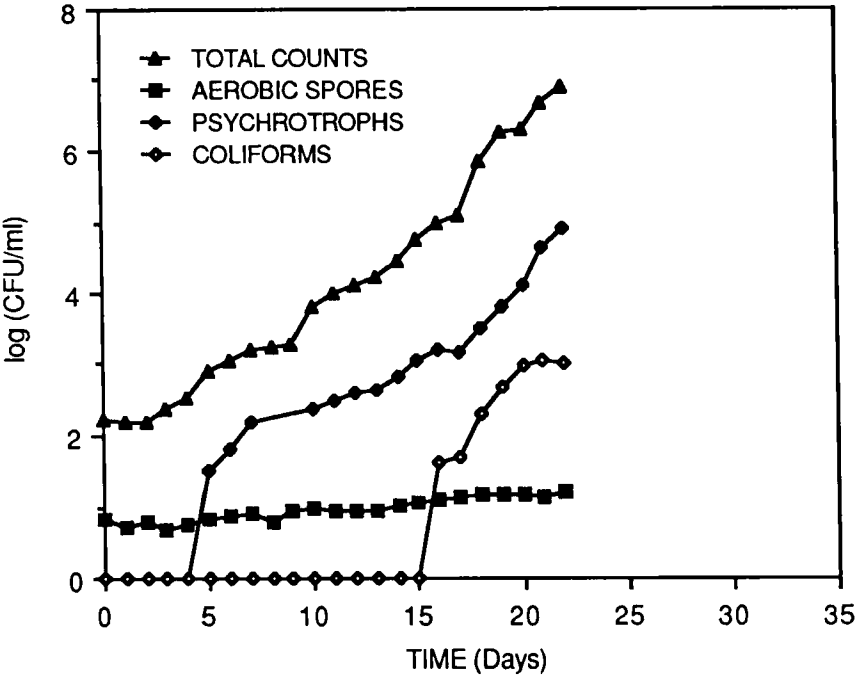

FIG. 3a. CHARACTERIZATION OF THE MICROBIAL GROWTH IN PASTEURIZED MILK FOR THE 5 °C STORAGE TREATMENT

Bacilli strains (Piggot and Coote 1976). Thus, a rich medium such as milk usually will not provide the nutritional impetus for spore development. The temperature-dependent lag which preceded the psychrotroph growth may reflect poor germination at low temperatures. Chung and Cannon (1971) reported lag times of 8–14 days for the outgrowth of *Bacillus* spores in milk held at 5 °C. Similarly, Mikolajcik and Simon (1978) showed that milk containing no detectable psychrotrophic Bacilli directly after pasteurization frequently contains high counts of these organisms after 2–4 weeks of storage at 7 °C.

Dahlberg (1946) argued that coliform bacteria were the most significant class of common milk contaminants because of their ability to multiply rapidly, and their severe health threat. In the present study coliforms were detected in the 5 °C samples, but failed to proliferate at both 0 °C and 10 °C. Coliform appearance at 5 °C was preceded by a 15 day lag, which suggests the need for recovery from injury. The near-freezing incubation of the 0 °C treatment probably inhibited coliform recovery (Dabbah and Moats 1968; Maxcy 1970), and the product stored at 10 °C most likely spoiled by the predominant bacteria before coliforms were able to repair pasteurization damage.

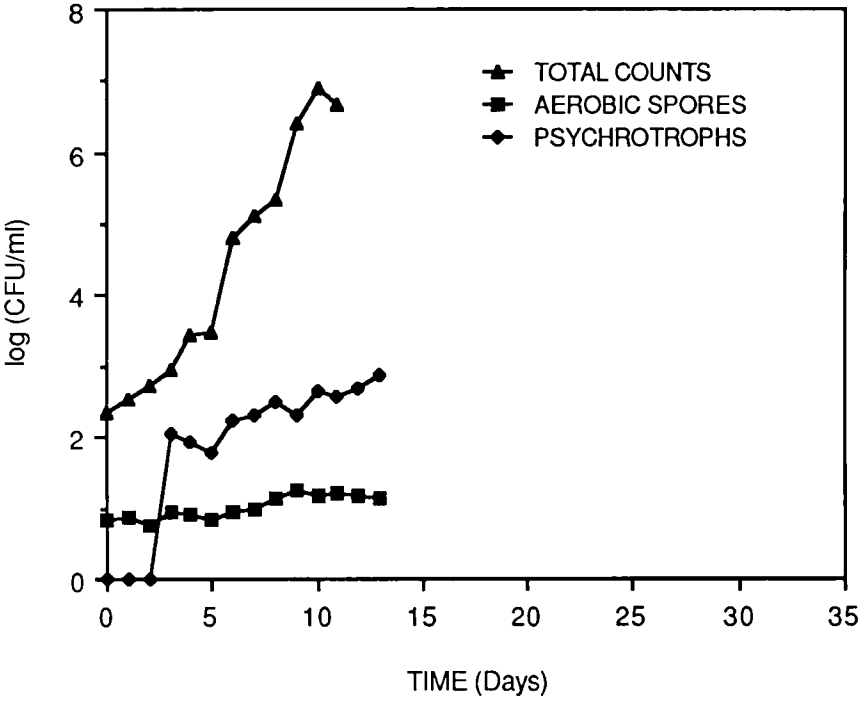

FIG. 4a. CHARACTERIZATION OF THE MICROBIAL GROWTH IN PASTEURIZED MILK FOR THE 10°C STORAGE TREATMENT

Time-Temperature Indicator Correlations

Responses of the Lifeline and I-POINT time-temperature indicators observed at the four different treatment conditions are shown in Fig. 2b, 3b, 4b and 5b. For proper comparison between indicator response and microbial growth, both microbial growth and indicator response must change consistently with time, and be consistently correlated with each other at different storage temperatures. Table 1 shows the linear correlation coefficients and levels of significance between storage time, indicator responses, and microbial counts at each constant temperature treatment. Because of the extended lag period and the variation in growth of heat damaged coliforms, no correlations between coliform growth and indicator response were attempted. Correlations between indicator response and counts of aerobic spore formers were also inappropriate, as neither spore former growth nor indicator response were consistently correlated with storage time.

Changes in the total counts were the only data which proved to be significantly correlated to indicator response at all three constant temperature storage conditions. Correlations between the LifeLine model 57 response and total counts

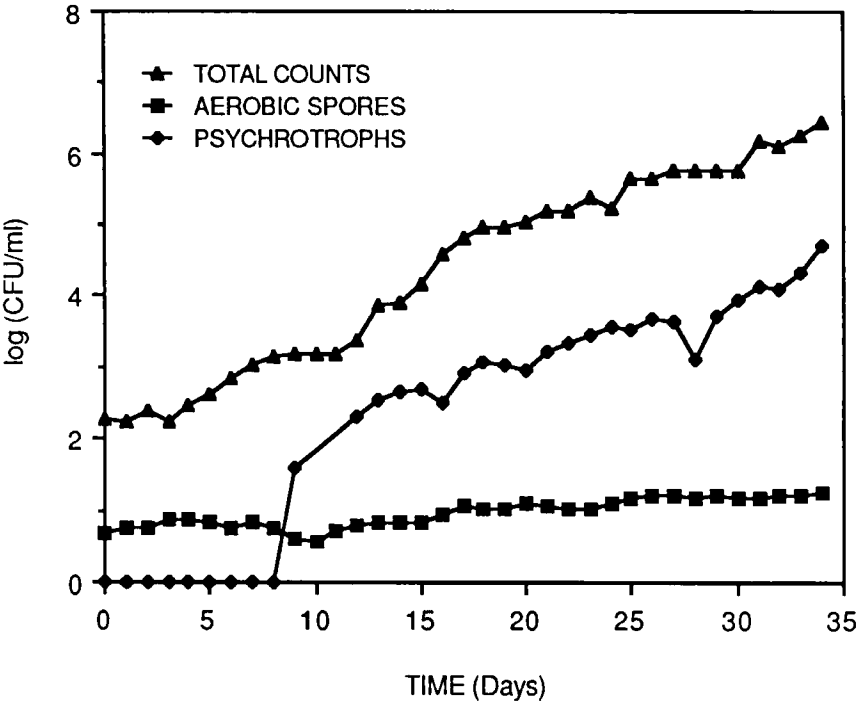

FIG. 5a. CHARACTERIZATION OF THE MICROBIAL GROWTH IN PASTEURIZED MILK FOR THE VARIABLE TEMPERATURE STORAGE TREATMENT

were significant ($p < 0.05$) at 10 °C, and were highly significant ($p < 0.001$) at 0 °C and 5 °C. The slight decrease in correlation coefficient significance at 10 °C was a result of fewer indicator inspections than at the other treatment conditions, thus reducing the number of degrees of freedom contributing to the calculation of the correlation coefficient.

Campbell *et al.* (1986) suggested that if consistent correlations exist at several isothermal storage conditions, regression analysis could be used to obtain a prediction equation to estimate food quality change based on indicator response. Figure 6 illustrates the correlation between total counts and LifeLine model 57 indicator response for the constant and variable temperature treatments. Regression analysis was used to construct 99% confidence limits between LifeLine 57 indicator response and normalized logarithm of total count CFU/mL collected at the constant temperature storage. Indicator response and total count data obtained from the variable treatment fall within the confidence limits of the prediction equation. This demonstrates the validity of the regression approach in estimating food quality changes from indicator response.

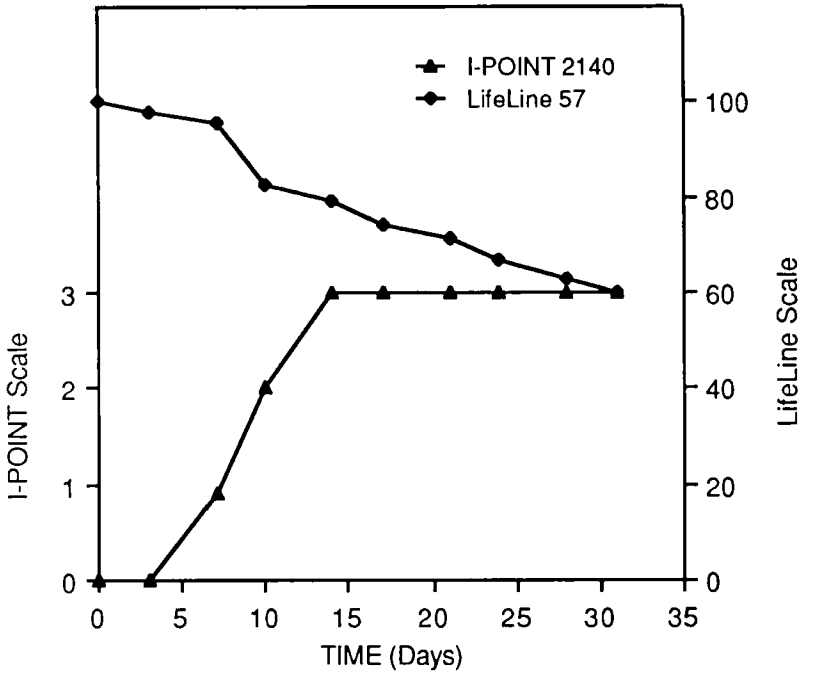

FIG. 2b. RESPONSE OF THE LIFELINE 57 AND I-POINT 2140 TIME-TEMPERATURE INDICATORS DURING THE 0°C STORAGE TREATMENT

Although psychrotrophic counts were not consistently correlated with indicator response, the length of the lag periods prior to the appearance of psychrotrophic growth might be an important determinant of shelf-life. The time required for injury recovery or spore germination is dependent on the storage temperature. Likewise, the I-POINT indicators initiate a discernible color change (the indicator response) only after a predetermined time and temperature combination has been achieved (Blixt and Tiru 1976). Thus, I-POINT indicators exhibit a time-temperature dependent lag prior to initiating a color change, much like the time-temperature dependent lag observed in the psychrotrophic bacteria counts. Figure 7 illustrates the relation between response lag of I-POINT model 2140 and the lag in psychrotrophic growth for all treatments. Because the response of the I-POINT model 2140 indicator also mimicked the delay in the growth lag in the variable treatment condition, it would appear that the I-POINT indicator might be particularly useful in situations where microbial spoilage in milk are typically the result of contamination by psychrotrophic *Bacillus* species.

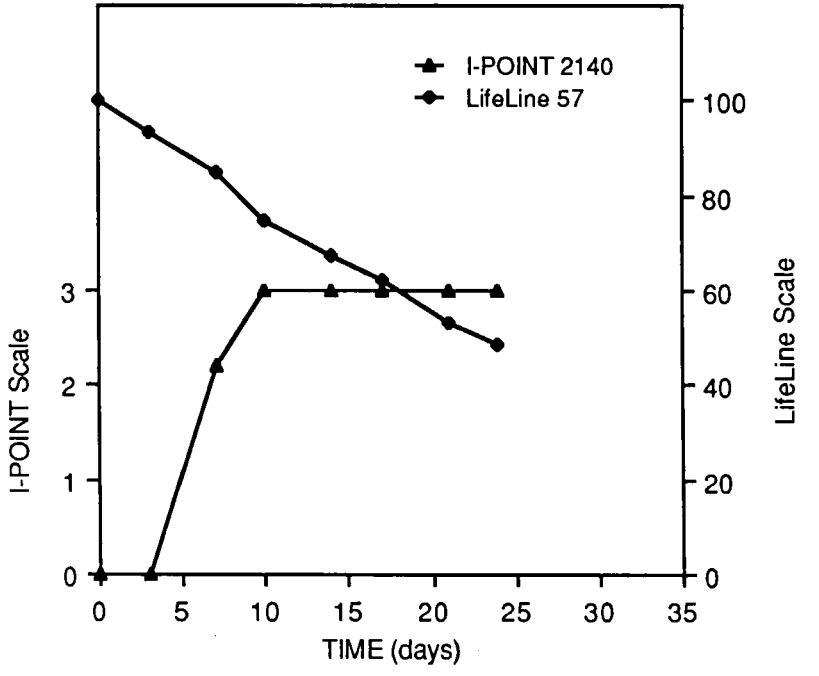

FIG. 3b. RESPONSE OF THE LIFELINE 57 AND I-POINT 2140 TIME-TEMPERATURE INDICATORS DURING THE 5°C STORAGE TREATMENT

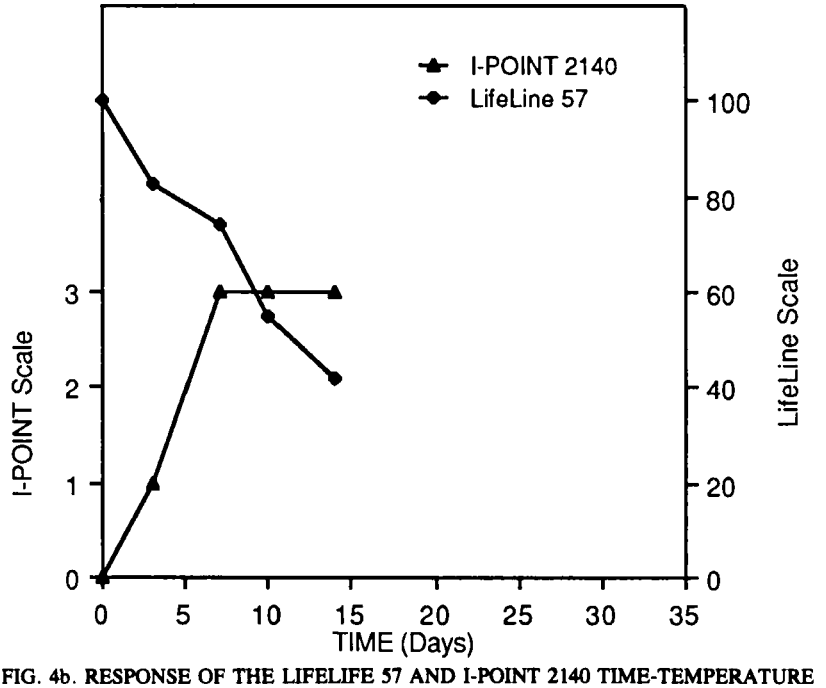

FIG. 4b. RESPONSE OF THE LIFELIFE 57 AND I-POINT 2140 TIME-TEMPERATURE INDICATORS DURING THE 10°C STORAGE TREATMENT

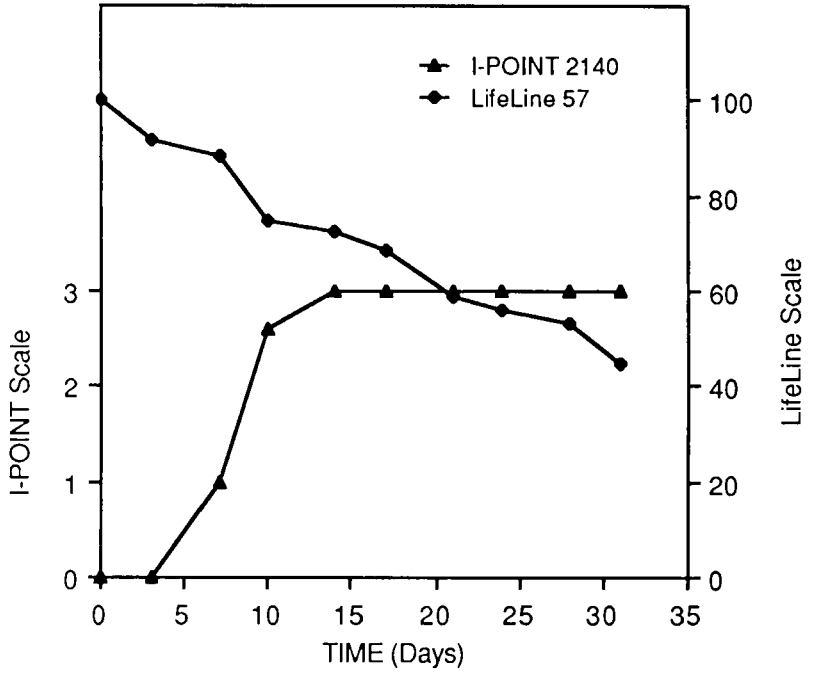

FIG. 5b. RESPONSE OF THE LIFELINE 57 and I-POINT 2140 TIME-TEMPERATURE INDICATORS DURING THE VARIABLE TEMPERATURE STORAGE TREATMENT

Results of this investigation illustrate the potential use for time-temperature indicators in estimating microbial growth in pasteurized whole milk. A single time-temperature indicator may not account for the differences in the quality and type of contaminants in milk from different processors; nor can a single indicator describe the complexity of microbial growth patterns arising from mixed populations incubated at changing temperatures. However, a combination of the different types of full-history time-temperature indicators could be used to estimate the presence and growth of spoilage organism within pasteurized milk.

TABLE 1.
CORRELATION COEFFICIENTS AND SIGNIFICANCE LEVELS OF CORRELATIONS BETWEEN STORAGE TIME, TIME-TEMPERATURE INDICATOR RESPONSE, AND SELECTED MICROBIAL COUNTS AT EACH CONSTANT TEMPERATURE TREATMENT

		Correlation Coefficients and Significance Levels					
		Total Counts		Spore Formers		Psychrotrophics	
	Time	0.951[a]	***	0.308	n.s.	0.923	***
		0.981[b]	***	0.918	**	0.965	***
		0.985[c]	*	0.948	n.s.	0.875	n.s.
I-POINT TTM	2140	0.905	***	0.094	n.s.	0.722	*
		0.794	*	0.910	**	0.908	**
		0.926	n.s.	0.825	n.s.	0.871	n.s.
	2180	0.828	**	0.295	n.s.	0.943	***
		0.890	**	0.901	**	0.848	*
		0.965	*	0.823	n.s.	0.715	n.s.
	2220	0.841	*	0.291	n.s.	0.951	***
		0.935	**	0.890	**	0.865	*
		0.989	*	0.921	n.s.	0.686	n.s.
	2340	0.680	*	0.244	n.s.	0.755	*
		0.871	*	0.663	n.s.	0.751	n.s.
		0.823	n.s.	0.888	n.s.	0.498	n.s.
LifeLine	57	-0.940	***	-0.171	n.s.	-0.897	***
		-0.973	***	-0.926	**	-0.963	***
		-0.962	*	-0.988	*	-0.893	n.s.

a correlation coefficient for 0°C storage treatment
b correlation coefficient for 5°C storage treatment
c correlation coefficient for 10°C storage treatment

Levels of significance designated as
* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$
n.s. not significant
$p > 0.05$

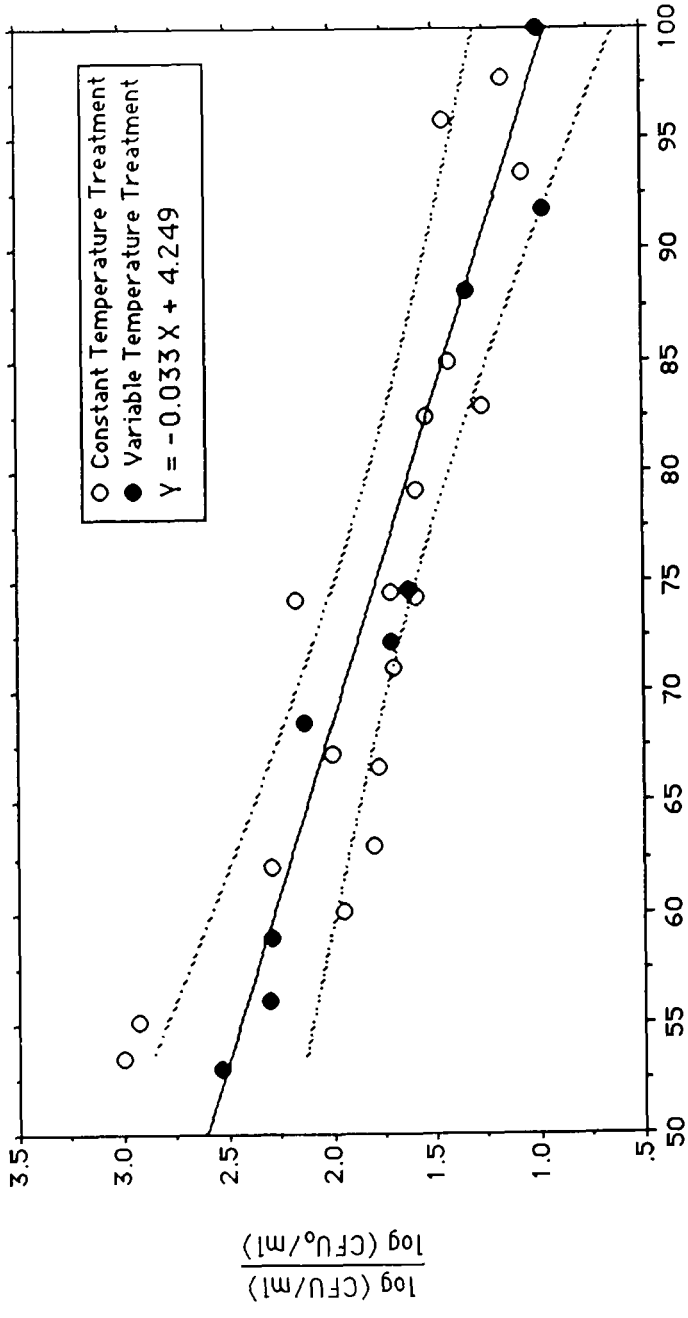

FIG. 6. PLOT OF NORMALIZED TOTAL COUNT ENUMERATION AND RESPONSE OF LIFELINE INDICATOR MODEL 57 FOR CONSTANT AND VARIABLE TEMPERATURE TREATMENTS

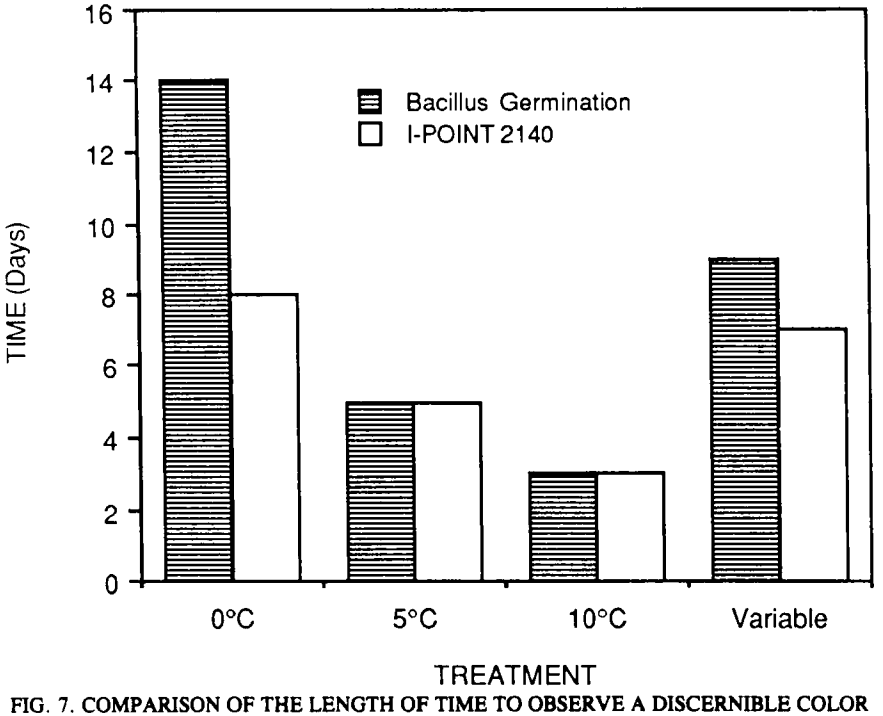

FIG. 7. COMPARISON OF THE LENGTH OF TIME TO OBSERVE A DISCERNIBLE COLOR CHANGE FROM 0 TO 1 (GREEN TO YELLOW) IN THE I-POINT MODEL 2140 AND DETECTION OF THE PRESENCE OF PSYCHROTROPHIC BACTERIA IN PASTEURIZED MILK

CONCLUSIONS

(1) Full-history time-temperature indicators which exhibit a delay prior to initiating a discernible response (e.g. the I-POINT type indicator) are suitable for estimating the time delay prior to psychrotrophic Bacilli spore germination in pasteurized milk.

(2) Full-history time-temperature indicators which respond in a continuous fashion (e.g. the LifeLine type indicator) are suitable for estimating growth of the total microbial population in pasteurized milk.

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