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Evaluation of a Rapid Impedimetric Method for Determining the Keeping Quality of Milk

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ABSTRACT

Shelf-life of 151 pasteurized milk samples was recorded and correlation coefficients calculated using various microbiological factors: standard plate count (SPC), psychrotrophic plate count (PPC), coliform count (CC), and the impedance response detection time (DT) with incubation at both 21 and 32°C. These data were obtained for milk samples on the day of pasteurization as well as 4 and 8 d thereafter. Various treatments (media, dilution factors, temperature and sample volume) were compared. Of the SPC, PPC, CC and DT taken on the day of pasteurization, only the DT achieved a significant correlation with shelf-life. A correlation coefficient of 0.55 was obtained for one treatment applied to 61 samples and correlation coefficients of 0.28 to 0.32 were obtained for several other treatments applied to the entire 151 samples. Values as large as these could occur by chance in uncorrelated data with $p<0.0005$. Thus, of the total 61 samples, 80% were correctly classified by the impedance detection time test. It is concluded that for prediction of shelf-life on the day of pasteurization, the impedance method is superior to the SPC and the PPC. In addition, the impedance method is more rapid, i.e., 14 h vs.2 d for the SPC and 10 d for the PPC.

Milk processors place a great deal of importance on code dates on milk cartons because of their economic impact. The "code date" is the date stamped on the package at the time of pasteurization and is the last day when the milk may be offered for sale.

The code date ranges from 7 to 14 d after pasteurization. It varies from dairy to dairy and is usually assigned after lengthy observations of the milk's movement through a particular dairy, rather than actual studies on the keeping quality of that particular batch of milk (*15*). In fact, Hankin et al. (*15*) have estimated that almost half of the milk samples would be unacceptable if they were allowed to reach the end of the code period on the supermarket shelf.

There has always been considerable interest in relating the initial microbial count of pasteurized milk to its keeping quality. The assumption is that the higher the initial count, the shorter will be the time that the milk will be acceptable to the consumer. However, initial bacterial counts (standard plate, coliform or psychrotrophic counts) have proved to be of little value in predicting the keeping quality of milk (*21-23,29*).

The Moseley test (*2,10*), which determines the standard plate count after storage of milk for 5 d at 45°F (7.2°C), is more widely accepted in the industry as a better indicator of the keeping quality of milk (*3,22-25,29,31*). However, the period of 7 d necessary to obtain results renders the Moseley test rather impractical for monitoring the quality of a perishable product with a shelf-life of 14 d.

Clearly, a need exists for a test that would provide reliable estimates of milk keeping quality within a time period which would allow for effective corrective measures. Impedance monitoring techniques have previously been used to estimate levels of microbial content in frozen vegetables (*16*), milk (*7,20*), and ground meat (*18*) in less than 24 h. The purpose of this research was to determine the feasibility of the use of impedance measurements to predict the keeping quality of milk, in comparison with the conventional time-consuming and laborious plating techniques for enumerating bacteria.

MATERIALS AND METHODS

Sample collection

A total of 151 samples of pasteurized whole milk were obtained from two local dairies and analyzed. Each sample consisted of two half-pint cartons collected from the dairy in the original carton. Day 0 analysis was made within 6 to 8 h after pasteurization. The milk cartons were refrigerated at 7°C and analyzed by the impedance method (DT) and by conventional microbiological techniques for standard plate count (SPC), coliform count (CC) and psychrotrophic plate count (PPC) and for keeping quality by flavor scoring according to the schedule outlined in Table 1.

Impedance measurements

Test conditions. The impedance monitoring instrument was the Bactometer 32 Microbial Monitoring System (Bactomatic, Inc., Princeton, NJ) and is described fully elsewhere (*5,6*). The system was operated at 2 KHz, gain 9, and the impedance changes automatically recorded every 3 s on a strip chart recorder. The time at which the impedance change becomes detectable is called the detection time (DT). For this study, DT was defined as the time required to produce an accelerating impedance change of 1.6% (2 channel widths of the strip chart record).

Impedance measurements were made at 32 and 21°C in two types of containers:

(a) Disposable plastic modules with 8 samples and 8 reference wells, each provided with vertical stainless steel electrodes (*6*). Each well had a nominal capacity of 2.0 ml. The reference well contained 2.0 ml of

TABLE 1. *Testing schedule for analysis of milk samples by microbiological tests (SPC, PPC and CC)[a], impedance detection times (DT), and flavor scoring (FS).*

Tests performed	Days after pasteurization 0	4	6	8	10	11	13	14
SPC	x	x		x	x			
PPC	x	x		x				
CC	x	x		x				
FS[b]			x	x	x	x	x	x
DT	x	x		x				

[a]SPC (standard plate count), PPC (psychrotrophic plate count) and CC (coliform count).
[b]Flavor scoring continued daily until spoilage occurred.

TABLE 2. *Typical values of SPC, PPC and CC on the day of pasteurization and 4 and 8 d thereafter for milk samples with short shelf-life (9 d), medium shelf-life (12-13 d) and long shelf-life (15-17 d).*

Sample No.	Shelf-life (d)	SPC (CFU/ml)[a] Day 0	Day 4	Day 8	PPC (CFU/ml)[a] Day 0	Day 4	Day 8	CC (CFU/ml)[a] Day 0	Day 4	Day 8
8	9	4×10^2	2×10^4	2×10^7	10	2×10^4	2×10^7	1	1	10
26	9	7×10^3	1×10^3	2×10^6	30	3×10^3	5×10^5	1	1	10^2
50	9	5×10^2	3×10^3	2×10^7	10	3×10^3	2×10^7	10	10	1
53	9	7×10^2	6×10^3	5×10^7	10	1×10^3	3×10^7	10	10	2×10^3
55	9	8×10^2	5×10^3	3×10^6	10	6×10^2	3×10^6	1	1	1
21	12	2×10^3	1×10^3	1×10^5	10	1×10^2	1×10^5	1	1	1
31	13	3×10^2	6×10^4	1×10^8	70	5×10^4	1×10^8	10	10	3×10^3
39	13	8×10^2	8×10^4	1×10^8	10	6×10^4	5×10^7	1	1	1
5	15	2×10^2	1×10^3	1×10^5	10	1×10	ND[b]	1	1	10
41	15	3×10^2	5×10^3	2×10^4	10	3×10^3	3×10^4	1	1	1
43	15	3×10^2	1×10^2	4×10^4	10	1×10^2	4×10^4	1	1	1
44	15	4×10^2	1×10^3	5×10^6	10	1×10^2	4×10^6	1	1	1
4	17	3×10^2	9×10^3	5×10^6	10	1×10^4	ND[b]	1	1	4×10^2

[a]SPC (standard plate count), PPC (psychrotrophic plate count) and CC (coliform count) are an average of duplicate plate counts.
[b]ND = no data.

sterile medium; the test well contained 1.0 ml of medium and 1.0 ml of the milk sample. Each milk sample was analyzed in quadruplicate and an average DT was obtained.

(b) Vials with vertical stainless steel electrodes. For each test vial containing 5 ml of sterile medium and 5 ml of milk sample, there was a corresponding reference vial containing 10 ml of sterile medium. Each milk sample was tested in duplicate and an average DT was determined.

Media. The media used were:

(a) Standard methods broth (BBL) with 0.5% sodium sulfate (SMB +).

(b) Trypticase soy broth (BBL) with 0.1% yeast extract (TSBY).

Sample preparation. An undiluted milk sample and a 1:10 dilution in TSBY or SMB + was inoculated into an equal volume of each of the above media to give final dilutions of 1:2 and 1:20.

Conventional microbiological procedures

Standard plate count (SPC). SPC counts were determined by the procedure recommended by the American Public Health Association (APHA) (2) using standard method agar (Gibco). The plate counts were done in duplicate using two or more serial decimal dilutions of milk sample. The plates were overlaid with agar before incubation at 32°C for 48 h to restrict the colony size of spreaders like pseudomonads. The average of duplicates determined the SPC.

Psychrotrophic plate count (PPC). PPC counts were determined using standard methods agar by the pour plate method without an agar overlay. The plate counts were made in duplicate using two or more serial decimal dilutions of each milk sample. The plates were incubated at 7°C for 10 d as recommended by APHA (2) and also at 21°C for 25 h as described by Oliveria and Parmelee (*21*). An average of the duplicates determined the PPC.

Coliform count (CC). CC determinations were made using violet red bile agar (BBL) by the standard APHA pour plate technique (2). The plates were incubated at 32°C for 24 h and an average of duplicates obtained.

Flavor scoring

Flavor scoring (FS) was done according to the method used by Nelson and Trout (*19*) and by Hankin et al. (*13,14*). A panel of at least three trained milk tasters graded the milk for flavor. A flavor score of 40 was considered excellent, whereas 35.5 or less was considered unsatisfactory and the milk sample was judged spoiled. Shelf-life was the number of days that a milk sample maintained a score greater than 35.5 as judged by two or more members of the taste panel.

RESULTS AND DISCUSSION

Relationship between SPC, PPC and CC with shelf-life

Milk samples were analyzed routinely for their microbiological counts on days 0,4 and 8 after pasteurization. Table 2 gives the values of SPC, PPC and CC of typical samples of milk with a minimum shelf-life of 9 d and a maximum of 17 d. No clear difference was apparent between the milk samples which lasted 9 d and those which lasted 17 d. (Day 0 SPC values in Table 2 are somewhat higher for milks lasting only 9 d than for those lasting 17

TABLE 4. *Coefficient of correlation between shelf-life measurements and detection times by the impedance technique under various test conditions.*

Test condition	Medium	Dilution	Total No. examined	Correlation coefficient
	TSBY	1:2	151	0.28[a]
32°C	SMB +	1:2	151	0.32[a]
(vials)	TSBY	1:20	61	0.55[a]
	SMB +	1:20	60	0.48[a]
	TSBY	1:2	151	0.13
32°C	SMB +	1:2	151	0.31[a]
(modules)	TSBY	1:20	62	0.37[a]
	SMB +	1:20	60	0.43[a]
21°C (vials)	SMB +	1:2	83	0.14
21°C (modules)	SMB +	1:2	122	0.12
	SMB +	1:20	41	0.21

[a]Significantly different from zero at the 0.01 level.

TABLE 3. *Coefficient of correlation between shelf-life (61 samples) and SPC, PPC and CC taken on the day of pasteurization and 4 and 8 d thereafter.*[a]

Microbiological assay	Day 0	Day 4	Day 8
SPC	0.03[b]	0.03	0.14
PPC	-0.01	0.00	-0.19
CC	-0.17	-0.01	-0.24

[a]These included results obtained by interpolation (on a logarithmic scale) for some readings that had to be done on weekends.
[b]Coefficient of correlation for the entire 151 samples was -0.05.

d. However, this trend was not borne out by the remainder of our samples.) Thus, these data agree with earlier findings (*15,23,25,29*) that suggest SPC, PPC and CC on the day of pasteurization do not correlate with the keeping quality of milk. Even with counts as high as 10^8 CFU/ml on day 8, some milk samples (#31 and #39) had an acceptable flavor which continued for an additional 5 d.

The SPC, PPC and CC counts determined 4 d and even 8 d after pasteurization did not correlate significantly with shelf-life either, as seen in Table 3. These results thus question the predictive value of the widely accepted Moseley test.

In the Moseley test, the pasteurized milk is stored at 7°C for 5 d before determination of SPC (*2*). In our study, the milk was stored for 4 and 8 d before SPC determinations. It is unlikely that the difference in storage period accounts for the observed lack of correlation, since other investigators have reported that the value of the Moseley test can be enhanced by increasing the storage period to 7 d. Randolph et al. (*24*) reported a coefficient of correlation of -0.028 between shelf-life and Moseley counts. As seen in Table 3, the best coefficient of correlation observed between shelf-life and either SPC or PPC on day 4 or 8 was -0.19, and this value was obtained with PPC rather than SPC. Few other studies have attempted to correlate Moseley counts with the actual keeping quality of milk, although the Moseley test as an indicator of contamination of psychrotophic bacteria appears well-documented (*3,24,25,31*). The rationale for the use of the Moseley test, as well as other tests that have been proposed more recently (*4,8,12,21,30*) as indicators of the keeping quality of milk, rests largely on the assumption that psychrotrophic bacteria are the most important determinants of post-pasteurization milk spoilage (*10,12,26,27,31*). While this assumption may be true, it is perhaps more correct to say that the shelf-life of milk is more directly influenced by the biochemical activity of the contaminating microflora than by the total number of bacteria, whether they are estimated by SPC, PPC or CC. For example, certain spoilage defects in milk have been associated with distinct bacterial species (*3,11,15,31*). A small number of such organisms which can bring about undesirable changes in milk will more directly affect the keeping quality of milk than will large numbers of organisms which grow well at the storage temperature, but produce no undesirable changes.

The lack of correlation between total bacterial numbers and the shelf-life of milk is best illustrated by the observation that flavor deterioration in milk stored at 7°C was not associated with any particular level of SPC. Rather, there was a direct relation between the number of days the milk was stored and the SPC, i.e., the greater the number of days after pasteurization, the higher the SPC. Similar results have been reported by others (*15*).

Relationship between DT and shelf-life

Correlation coefficients between shelf-life and DT are shown in Table 4. The experimental conditions included two dilutions of milk (1:2 and 1:20), two media (SMB + and TSBY), two incubation temperatures 32 and 21°C), and two different containers for measuring impedance detection time (vials with 10.0 ml capacity and modules with 2.0 ml capacity). Preliminary experiments with undiluted milk proved unsatisfactory because of delayed and attenuated impedance responses.

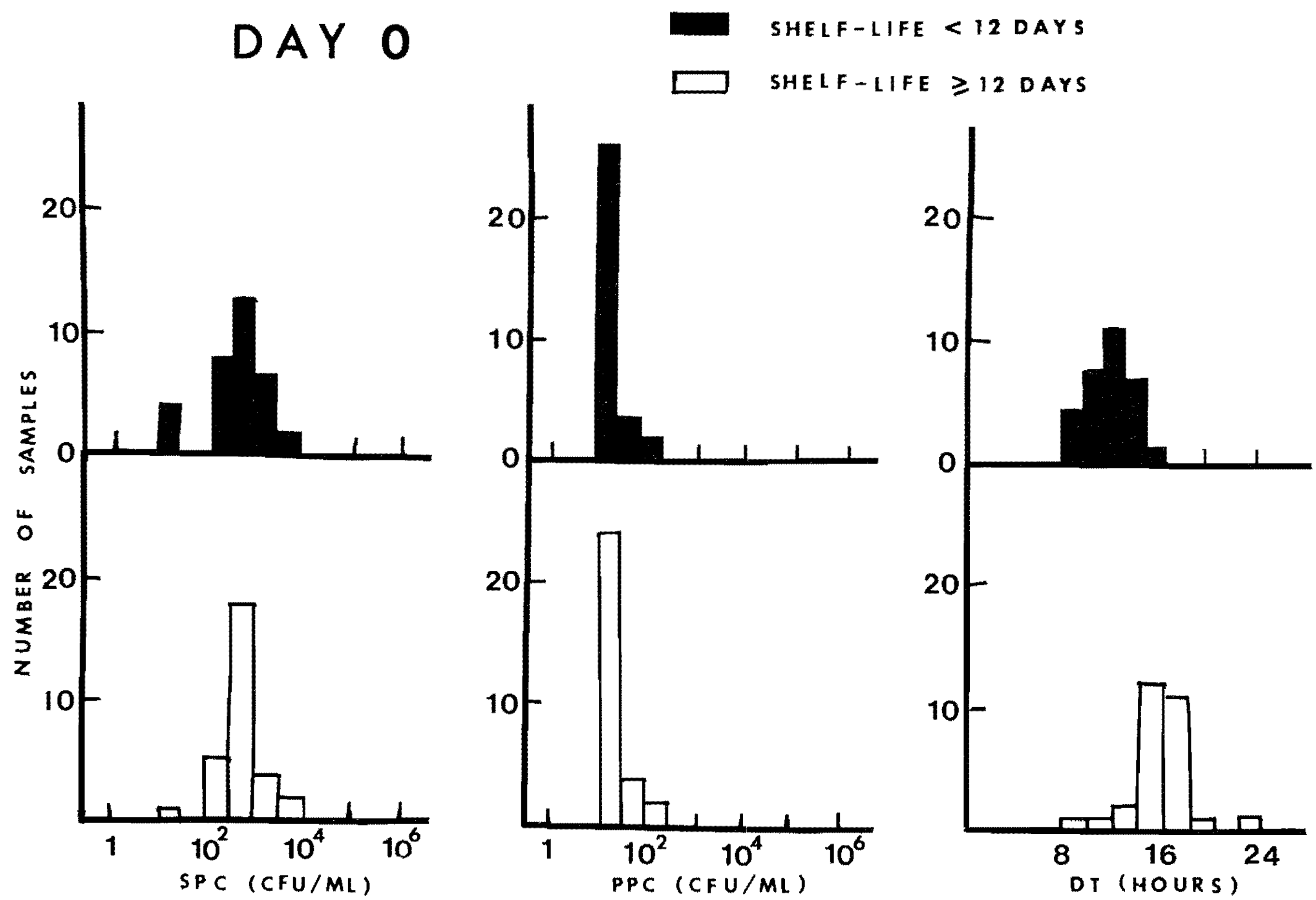

Figure 1. *Histograms comparing the distribution of SPC (standard plate count), PPC (psychrotrophic plate count), and DT (impedance response detection time) taken on the day of pasteurization (day 0) for milks having shelf-lifes less than 12 d vs. milks having shelf-lifes of 12 d or more.*

The positive correlation coefficients indicate that milk samples with short shelf-lifes tend to produce early impedance response detection times and samples with long shelf-lifes tend to produce late detection times. It can be seen that correlation coefficients significantly different from zero at the 0.01 level were obtained between shelf-life and impedance response detection times with incubation at 32°C. Of the 8 experimental conditions at 32°C incubation, 7 produced significant correlation coefficients at the 0.01 level. The best correlation coefficient between impedance measurements and shelf-life was obtained when 5 ml of a 1:10 dilution of milk was inoculated into an equal volume of TSBY medium and incubated at 32°C. However, it should be noted that aside from the 1:2 dilution in TSBY in modules, which was inferior, there was no clear superiority of any one of the 32°C-test conditions over any other when restricted to the same 61 samples.

A graphic representation of the SPC, PPC and DT data is shown in Fig. 1, 2 and 3. Figure 1 shows histograms of the SPC, PPC and DT for two groups of milk samples, those lasting less than 12 d ("short shelf-life") and those lasting 12 or more days ("long shelf-life"). The 12-d demarcation between "short shelf-life" and "long shelf-life" was chosen because the milk samples were approximately equally distributed on either side of the 12-d limit. As seen in Fig. 1, the distribution of short-life samples falls over the same SPC range as the long shelf-life samples. This clearly illustrates an inability to determine shelf-life of milk on the basis of SPC. Only when graphed against detection time is a shift apparent between the short shelf-life and the long shelf-life distributions. Figures 2 and 3 show the comparable distributions for SPC, PPC and CC on days 4 and 8. Again, no shift is apparent.

TABLE 5. *Distribution of 61 samples in a two-category classification scheme using a detection time cutoff of 13.5 h to predict if milk samples keep for 12 d.*

Shelf-life	Detection time	
	<13.5 h	≥13.5 h
<12 days	24 samples	8 samples
≥12 days	4 samples	25 samples

These results suggest that under certain conditions, changes in the electrical impedance of the medium may reflect the biochemical changes in the milk that ultimately result in flavor deterioration. It is known that many enzymes, including those of psychrotrophic bacteria, have temperature optima around 37°C (*1,31*). This may account for the poor results obtained with impedance measurements at 21°C. None of the three 21°C-treatments yielded a correlation coefficient significantly different from zero at the 0.01 level. The more significant correlation coefficients obtained with diluted milk could possibly reflect interference

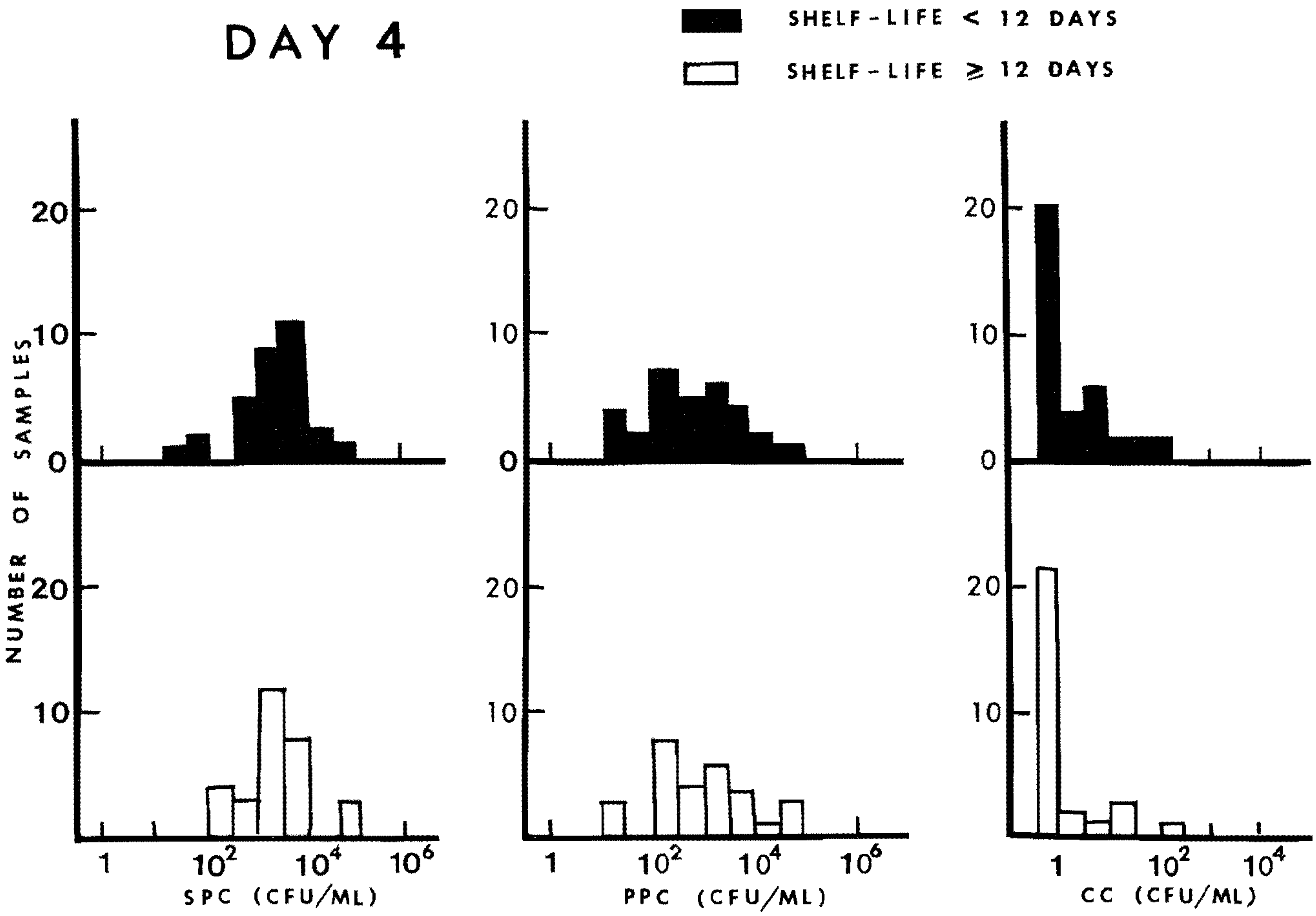

Figure 2. *Histograms comparing the distribution of SPC (standard plate count), PPC (psychrotrophic plate count), and CC (coliform count), taken 4 d after pasteurization (day 4), for milks having shelf-lifes less than 12 d vs. milks having shelf-lifes of 12 d or more.*

from preexisting endogenous enzymes in the milk, as well as those resulting from the microflora of the raw milk (*23*).

Based on the results obtained with the 61 samples tested under the optimum conditions, a simple and rapid test can be devised to predict the shelf-life of milk. As shown in Table 5, milk having DT≥13.5 h generally lasted 12 d or more and those having a DT<13.5 h usually spoiled within 11 d. Of the 28 milk samples with detection times before 13.5 h, 24 (86%) spoiled within 12 d. Of 33 milk samples with detection times after 13.5 h, 35 (76%) lasted 12 d or more. Thus, of the total 61 samples, 49 (80%) were correctly classified as to their shelf-life by the 13.5 h-impedance detection time test.

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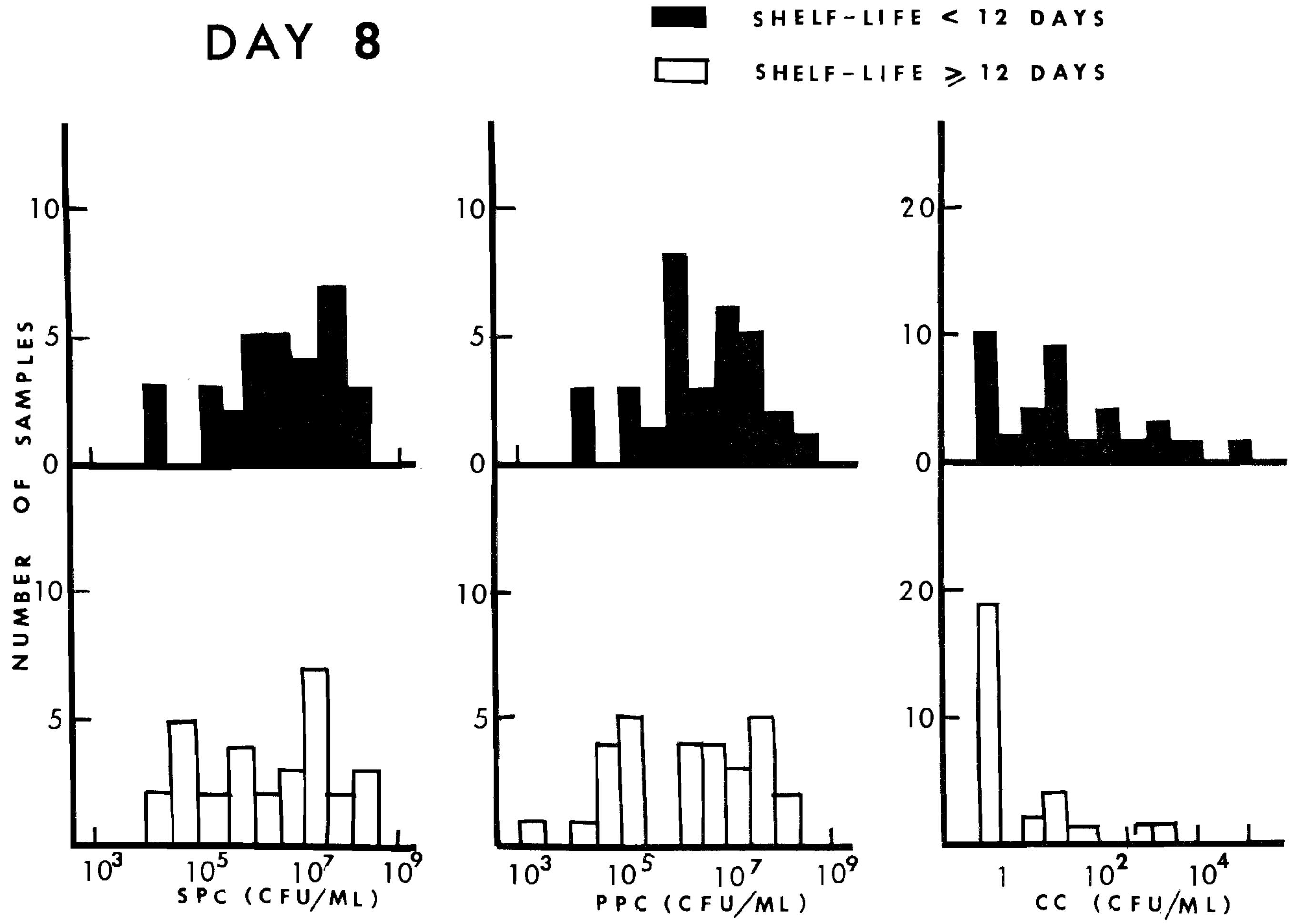

Figure 3. *Histograms comparing the distribution of SPC (standard plate count), PPC (psychrotrophic plate count), and CC (coliform count) taken 8 d after pasteurization (day 8) for milks having shelf-lifes less than 12 d vs. milks having shelf-lifes of 12 d or more.*

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