Application of Bioluminescence to Rapid Determination of Microbial Levels in Ground Beef

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ABSTRACT

The relationship between microbial ATP measurements and aerobic plate counts (APC's at 35, 20 and 7°C) was investigated for 75 ground beef samples. Samples (n = 27) were obtained from several local retail markets in one experiment, and ground beef samples obtained from a single, processing facility were sampled throughout 15 d of storage at 1°C for a total of 48 samples in another experiment. Bioluminescent assay time for a given sample was less than 1 h. The correlation coefficient (r) between \( \log_{10} \) microbial ATP and \( \log_{10} \) APC (20°C) per g was 0.86 and 0.99 for retail and single source samples, respectively. Differences between actual \( \log_{10} \) microbial ATP and corresponding values predicted by linear regression equations were \( \leq \log_{10} 0.5 \) for 25 of 27 retail samples and 48 of 48 single source samples. Variation was noted in values of ATP per bacterial cell and relative bioluminescent quenching (ATP per relative light unit, RLU) for most retail samples and for single source samples having low APC (20°C) levels (\( \leq \log_{10} 7.0 \)).

Conventional microbiological analyses such as the standard plate count are laborious and require incubation times of 24 to 72 h. This time element can delay detection of unacceptable raw materials, insanitary conditions or other microbial problems affecting product safety and subsequent initiation of corrective action by food processors. Development of rapid, sensitive and accurate methods for detecting and/or enumerating viable microorganisms in foods has received considerable attention over the past decade. These include biophysical methods based upon microbial growth or metabolism in an appropriate growth medium, such as radiometry (14,21), impedance (9) and microcalorimetry (14), all of which require 3 to 8 h before achieving results. More rapid techniques for indirect enumeration of microbial levels within 1 h are based upon detection of specific microbial cell components and are exemplified by the Limulus lysate endotoxin test for estimation of gram-negative bacterial levels (12) and the bioluminescent adenosine triphosphate (ATP) assay (17) for measurement of microbial ATP or total viable microbial levels (6,7,15). Each of these rapid methods has limitations in sensitivity, accuracy, type of sample material to which they can be applied, and in their resultant correlation to a total, viable microbial count, all of which must be evaluated and considered for specific applications to microbial food analyses.

Measurement of microbial ATP has been applied to enumerating bacterial populations in water (7,15), wastewater (16,27), marine environments (10), dental plaque (20), urine (4,18,24,26), milk (2), brewer processes (11) and various foods, including fresh meats (1,3,13,22,23,29); Littel et al., Abstr. 44th Annu. IFT Meet. 1984. No. 236, p. 139; Cook et al., Abstr. 44th Annu. IFT Meet. 1984, No. 237, p. 140). One universal difficulty which has been encountered in the application of this method to foods and other materials has been interference by non-microbial sources of ATP (2,4,22,29). Selective measurement of microbial ATP in the presence of nonmicrobial ATP has been accomplished with reasonable success by selective extraction and enzymatic destruction of non-microbial ATP followed by extraction and assay of microbial ATP (3,4,18,24,26) or by physical separation of microorganisms from sample material followed by extraction and assay of microbial ATP (23). In addition, the recent introduction of simple and rapid commercial bioluminescent ATP assay systems having standardized reagent kits and sensitive instrumentation has made application of these methods more feasible for routine microbiological monitoring of foods.

Bioluminescent techniques have been specifically applied to estimating microbial levels on meat surfaces with varying degrees of success (2,3,13). Baumgart et al. (1) reported some interference by nonmicrobial ATP in meat surface samples, whereas Carlier et al. (3) found a linear relationship between \( \log_{10} \) relative light units (RLU) and \( \log_{10} \) colony count of beef carcass surfaces with correlation coefficients of 0.79 to 0.80 using an enzymatic procedure for elimination of nonmicrobial ATP. Using a rapid procedure for physical separation of bacteria from meat tissue homogenates for selective assay of bacterial ATP, Stannard and Wood (23) reported a linear relation-
ship between log_{10} microbial ATP content and log_{10} colony-forming units (CFU) in various fresh meat tissues, with a correlation coefficient of 0.94.

This study was undertaken to investigate the application of a commercially available, rapid bioluminescent ATP assay system, including a simple procedure for selective extraction and enzymatic elimination of non-microbial ATP, for estimating total microbial levels in ground beef. Microbial ATP and RLU measurements were compared to aerobic plate counts (APC's at 35, 20 and 7°C) in retail samples from several sources and in samples from a single, large processor.

MATERIALS AND METHODS

Meat samples

In one experiment, fresh ground beef samples (n = 18) were purchased from several local retail markets over a 2-month period. Samples were transported to the laboratory in refrigerated containers and maintained at 1 to 2°C in the laboratory until the time of analysis. All samples were analyzed within 6 h of receipt and some (n = 9) were held at 1 to 2°C for 3 to 5 d to obtain higher microbial levels upon reexamination. A total of 27 sample units were examined. In another experiment, approximately 1500 g of fresh ground beef from each of six different processing lots were obtained immediately following fabrication from a large meat processor. These samples were transported to the laboratory in a refrigerated container within 2 h and packaged in styrofoam trays overwrapped with polyvinylchloride film while maintaining the integrity of samples from each lot. Samples were stored at 1°C and one sub-sample from each lot (n = 6) was analyzed initially and at 2 to 3-d intervals (n = 8) over 15 d of storage to obtain a total of 48 sample units.

Sample preparation

Ground beef samples were prepared for analysis by blending 50 g of sample in 450 ml of sterile phosphate-buffered diluent for 2 min (28). For aerobic plate counts, serial dilutions were prepared from the 1:10 homogenate and 0.5-ml portions were plated on prepoured plates of plate count agar (Difco) using the surface plate method. Duplicate plates from appropriate dilutions were incubated at 35°C for 48 h, at 20°C for 5 d or at 7°C for 10 d.

Materials for ATP assay

The Lumac bioluminescent ATP assay system (Medical Products Division/3M, St. Paul, MN), including standardized bioluminescent reagents and the Biocounter M2000 instrument, was used for all bioluminescent analyses. Reagents included luciferin-luciferase mixture (Lumit-PM), nucleotide-releasing agent for bacterial cells (NRB), nucleotide-releasing agent for somatic (non-microbial) cells (NRS), ATPase (Somase), ATP standard and Lumit Buffer (0.025 M Hepes, pH 7.75). Reagents were stored, prepared and used according to recommendations of the manufacturer. Luciferin-luciferase and ATPase mixtures were used immediately after preparation or portions were frozen at -20°C for later use. ATP standard (10 µg) was dissolved in 20 ml of sterile double distilled water and used immediately or portions were frozen at -20°C until needed. All reagents were kept at 5°C or below, except during actual use when appropriate amounts were held at room temperature (25°C). A mixture consisting of 10 µl reconstituted ATPase in 1.0 ml NRS (NRS/Somase) was freshly prepared for extraction and destruction of nonmicrobial ATP before each series of analyses. Plastic cuvettes (lumacuvette) were used for bioluminescent measurements and sample preparation. The Biocounter M2000 instrument provided an automatic injection of 0.1-ml portions of Lumit-PM reagent into sample cuvettes in a sealed reaction chamber with a digital readout of relative light units (RLU) ranging from 0 to 200,000 based upon an automatic integration of light emission over 10 s following a 2-s delay as suggested by the manufacturer.

ATP assay

One-ml portions of sample homogenate were clarified for subsequent bioluminescent analysis by drawing the homogenate through 1-ml pipettes fitted with sterile, disposable "filter tips" capped with 100-µm pore size polyethylene mesh (Spectramesh, Spectrum Medical Industries, Los Angeles, CA). Construction of disposable "filter tips" was similar to that described by Peterkin and Sharpe (19). Methods for microbial ATP determinations are outlined in Figure 1. For each sample homogenate, duplicate or triplicate 0.2-ml portions of filtered homogenate were assayed as indicated. Reagent blanks of sterile diluent were likewise prepared in parallel for each series of analyses. After the 45-min treatment for elimination of non-microbial ATP, three 50-µl portions from each sample mixture were each transferred to plastic cuvettes. To the first cuvette, 100 µl of NRB was added and the cuvette gently mixed by hand for 10 s to extract microbial ATP. The cuvette was then placed in the measuring chamber of the instrument, the injector head closed, and 100 µl of Lumit-PM automatically injected into the cuvette. After a 2-s delay, the resulting light emission was automatically integrated over 10 s as suggested by the manufacturer and displayed as RLU. Sample preparations producing more than 200,000 RLU were diluted 1:10 with sterile double distilled water, treated with NRB and remeasured as described. To the second cuvette, 100 µl of NRS was added instead of NRB and analyzed as previously described to obtain a blank value for any residual, nonmicrobial ATP which might remain after the NRS/Somase treatment. The NRS blank value in RLU was subtracted from the NRB value to obtain the net RLU associated with microbial ATP. The third cuvette was used for internal standardization with ATP standard for conversion of
RLU values to ATP content. Internal standardization was deemed necessary to correct for differences in sample composition, i.e., color, turbidity, inhibitor and activator, which might contribute to quenching of the luminescent reaction. To the third cuvette, 100 µl NRB and 10 µl of a freshly prepared ATP standard solution were mixed and analyzed as indicated. Dilutions of ATP standards were prepared in sterile double distilled water to obtain RLU values 2 to 5 times that provided by the sample alone with NRB. The quantity of ATP/RLU in femtograms (fg, 10^-15 g) for each sample was calculated as summarized below:

\[ \text{fg ATP added} = \frac{\text{RLU}_a - \text{RLU}_b}{k} \]

\[ \text{fg ATP/50 µl portion} = k \times (\text{net RLU, sample alone}) - (\text{fg ATP, reagent blank}) \]

\[ \text{fg ATP/g sample} = \frac{\text{fg ATP/50 µl portion} \times \frac{1000 µl}{0.6 ml} \times \frac{0.2 ml}{\text{dilution, NRS/somase}} \times \frac{500 ml}{50 g}}{\text{dilution, sample homogenate}} \]

where \( \text{RLU}_a \) = RLU of cuvette containing sample + ATP standard, \( \text{RLU}_b \) = RLU of cuvette containing sample alone, and net RLU of sample = NRB - NRS measurement. The ATP contributions of reagent blanks were subtracted from respective sample ATP contents to obtained net ATP values.

Based upon internally standardized reagent blanks with standard ATP concentrations ranging from \( 5.0 \times 10^4 \) to \( 2.5 \times 10^6 \) fg per 50-µl portion, a linear relationship between log ATP (fg per g) or net RLU (per 50-µl portion) and log ATP (fg) per sample was obtained. The following regression equation was obtained:

\[ y = 0.97x - 1.05 \]

where \( y = \log_{10} \text{RLU} \) and \( x = \log_{10} \text{ATP} \) (fg).

RESULTS AND DISCUSSION

Regression equations and correlation coefficients \((r)\) between \( \log_{10} \text{ATP} \) (fg per g) or net RLU (per 50-µl sample portion) and \( \log_{10} \text{APC} \) 35, 20 or 7°C (per g) for ground beef samples are presented in Table 1. Correlations were higher with APC (20°C) and APC (7°C) than with APC (35°C) for both groups of samples. These results indicate a more representative and quantitative estimate of the predominant psychrotrophic microflora of refrigerated fresh beef by APC (20°C) or APC (7°C) than by APC (35°C). The more consistent relationship between APC (20°C) or APC (7°C) and ATP or RLU values is also illustrated in Figure 2 which plots changes in these measurements as a function of storage time for the single source samples. Correlation coefficients for ATP vs. APC (20°C) were much higher for the single source than retail samples, with values of 0.99 and 0.86, respectively, for retail and single source samples. The correlation coefficient between ATP and APC (20°C) for all 75 samples combined was 0.98.

The relationship between microbial ATP content and APC (20°C) for retail ground beef samples is more clearly illustrated by a scattergram (Figure 3). The range of APC (20°C) was relatively narrow (\( \log_{10} 6.5 \) to 9.0 CFU/g), with 23 of 27 samples having APC (20°C) between \( \log_{10} 7.0 \) and 8.5 CFU/g. Correlation between ATP values and APC's (Table 1) were only slightly higher than that between corresponding RLU and APC's despite correction for bioluminescent quenching implicit in ATP values. Correlation between ATP content and APC (20°C) and between net RLU values and APC (20°C) was higher than that reported for meat surfaces (3) and slightly less than that reported for various meat tissues (23). Differences between actual \( \log_{10} \text{APC} \) (20°C) values and those predicted by the regression equation were \( \leq \log_{10} 0.5 \) for 25 of 27 samples and \( \leq \log_{10} 0.3 \) for 22 of 27 samples. Because 26 of 27 retail samples had APC (20°C) in excess of \( \log_{10} 7.0 \), an experiment was designed to analyze a group of samples with a broader range of APC's and some samples having lower APC's. This was accomplished by obtaining fresh ground beef from a single, large processing operation as previously described. In addition, these samples would be subject to less variation in terms of composition, age, handling, temperature exposure and microbial flora, and exemplify the potential application of bioluminescent methods to microbiological assessments in a typical processing operation. The relationship between microbial ATP and APC (20°C) for the single source samples is illustrated in Figure 4. A much higher degree of correlation \((r = 0.99)\) was noted between ATP and APC (20°C) determinations for these samples than for retail samples (Table 1). Correlations between ATP or net RLU measurements and APC (20°C) were also higher than those reported for meats in recent studies (3,23). Differences between actual and predicted APC (20°C) values were \( \leq \log_{10} 0.5 \) for 48 samples and \( \leq \log_{10} 0.3 \) for 43 of 48 samples. Correlations between net RLU and APC values were equivalent to those between ATP and APC values. These results may reflect low variation in sample composition and/or quenching for most samples as will be further discussed. Higher correlations obtained between APC and bioluminescent measurements with single source than retail samples could also be attributed in part to the larger range of APC values used for the regression analyses of single source samples. However, correlations between ATP and APC (20°C) values for single source samples over a smaller range of APC (20°C), similar to that of retail samples \((\log_{10} 6.5 \) to 9.0\), was 0.98. In addition, these data indicate that net RLU values alone may provide accurate estimates of microbial levels for most routine quality assessments using this bioluminescent technique and further reduce the time and effort of analysis.

To assess the relationship between bioluminescent and APC determinations for a larger and more diverse group of ground beef samples, data from both retail and single source samples were combined and analyzed (Table 1). Regression equations and correlations were very similar.
TABLE 1. Statistical analyses of ATP, RLU and APC determinations for ground beef samples.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>n</th>
<th>Regression equation</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single processor</td>
<td>48</td>
<td>ATP = 0.77(APC35) + 3.07</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ATP = 0.69(APC20) + 3.00</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ATP = 0.69(APC 7) + 3.09</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RLU = 0.88(APC35) - 1.92</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RLU = 0.79(APC20) - 1.99</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RLU = 0.78(APC 7) - 1.89</td>
<td>0.99</td>
</tr>
<tr>
<td>Retail samples</td>
<td>27</td>
<td>ATP = 0.96(APC35) + 1.49</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ATP = 0.82(APC20) + 2.00</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ATP = 0.79(APC 7) + 2.25</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RLU = 0.85(APC35) - 2.03</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RLU = 0.75(APC20) - 1.81</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RLU = 0.73(APC 7) - 1.62</td>
<td>0.86</td>
</tr>
<tr>
<td>All samples</td>
<td>75</td>
<td>ATP = 0.77(APC35) + 2.97</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ATP = 0.70(APC20) + 2.93</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ATP = 0.69(APC 7) + 3.02</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RLU = 0.87(APC35) - 1.97</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RLU = 0.79(APC20) - 2.02</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RLU = 0.78(APC 7) - 1.92</td>
<td>0.98</td>
</tr>
</tbody>
</table>

*Number of sample units.

bATP values expressed as log_{10} ATP (fg)/g of sample; net RLU expressed as log_{10} RLU; APC 35, 20 or 7 values expressed as log_{10} CFU/g.

Figure 2. Changes in microbial ATP, RLU and APC’s of ground beef from a single processor during storage at 1°C. Data points represent mean of six samples.

Figure 3. Scattergram relating log_{10} microbial ATP content and log_{10} APC (20°C) for 27 retail ground beef samples.

To those obtained with the single source samples due to the experimental design as well as the larger range and number of values with the single source samples. These data also imply that the regression equations obtained with the single source samples using this system could be applied to various other ground beef samples with reasonable accuracy. Differences between actual and predicted APC (20°C) for either RLU or ATP regression equations were ≤log_{10} 0.5 for 71 of 75 samples. The relationship between ATP and APC and between RLU and APC was also similar in terms of accuracy (Table 1).

ATP/CFU and/or ATP/cell values (fg ATP/CFU) were calculated for each sample and the means of samples falling within various levels of APC (20°C) are presented in Table 2. For retail samples, ATP/CFU values were subject to wide variation (0.6 to 17.1 ATP/CFU), with ATP/CFU values generally being inversely related to APC levels. For single source samples, there was a consistent inverse relationship between ATP/CFU values and APC levels up to log_{10} 7.0, which is also evident in Fig-
ure 2. There was also considerably more variation in ATP/CFU values for samples having APC (20°C) of \( \leq \log_{10} 6.0 \) (12.7 to 80.4) than for samples having APC (20°C) between \( \log_{10} 7.0 \) and 9.0 (2.3 to 3.6). Stannard and Wood (23) also found highest ATP/CFU values and variation at CFU levels of \( \log_{10} 5.0 \) to 6.0 in fresh beef, although ATP/CFU values were generally much lower than those obtained in this study. ATP/CFU values were also reported by Cook et al. (Abstr. 44th Annu. IFT Meet. 1984, No. 237, p. 140) to decrease with increasing APC’s in ground beef during storage. ATP/CFU values for most samples in this study were comparable to ATP/cell content of bacteria reported in the literature, with 20 of 27 retail and 31 of 48 single source samples having ATP/CFU values \( \leq 5.0 \) fg ATP/CFU. Average ATP/bacterial cell values of 0.5 and 2.0 fg have been used for enumerating mixed microbial populations in the environment (7,23) and clinical specimens (4,26), respectively, but reported ATP/cell values vary from 0.01 fg for Flavobacillus proteus (11) to 6.5 fg for Chromobacterium maximum (8). The ATP content of bacterial cells also varies with the physiological state of the bacteria (4,25) and an activation period of at least 30 min has been recommended for equilibration of ATP/cell levels before bioluminescent measurement (25). The 45-min NRS/Somase incubation used in this technique may afford consistent equilibration of the physiological state of microorganisms in ground beef samples.

The accuracy of bioluminescent estimation of microbial levels in foods or other materials is generally predicated upon low variation in ATP/CFU values, particularly if conversion of ATP measurement to CFU is based simply upon dividing ATP values by an average ATP/CFU value. However, the results of this study and that of Stannard and Wood (23) indicate that estimates based upon regression equations over a broad range of CFU values are very accurate despite variation in ATP/CFU values because the tendency of ATP/CFU values to decrease with increasing microbial levels is accommodated by the regression. Excessively large and relatively variable ATP/bacterial cell values encountered with samples having APC’s \( \leq \log_{10} 6.0 \) in this and another recent study (23), could be attributed to microbial ATP levels approaching the sensitivity limits of the instrument and/or method as suggested by Stannard and Wood (23). ATP levels near the sensitivity of the technique, contributions to light emission by background noise of the instrument, reagents and residual, nonmicrobial ATP may have disproportionately large effects on measurements. Additional sources of error could be associated ATP/cell variation due to clumping errors in colony counting, failure of some bacteria to form colonies on recovery medium and differences between the taxonomic profile and/or physiological state of the microflora among samples.

Two factors which may influence the accuracy and sensitivity of bioluminescent methods are bioluminescent quenching (Table 3) and nonmicrobial contributions to light emission (Table 4). The relative degree of quenching expressed as fg ATP (ATP standards) per RLU was

<table>
<thead>
<tr>
<th>Range of CFU/g (^a)</th>
<th>Retail samples</th>
<th>Single processor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \log_{10} )</td>
<td>ATP/CFU(^c)</td>
</tr>
<tr>
<td></td>
<td>CFU/g</td>
<td></td>
</tr>
<tr>
<td>4.5-6.0 range</td>
<td>0</td>
<td>---(^d)</td>
</tr>
<tr>
<td>6.0-7.0 range</td>
<td>1</td>
<td>4.71</td>
</tr>
<tr>
<td>7.0-8.0 range</td>
<td>16</td>
<td>5.16 ± 3.44</td>
</tr>
<tr>
<td>8.0-9.0 range</td>
<td>10</td>
<td>2.98 ± 1.92</td>
</tr>
<tr>
<td>&gt;9.0 mean range</td>
<td>0</td>
<td>---</td>
</tr>
</tbody>
</table>

\(^a\) Expressed as \( \log_{10} \) CFU/g of APC (20°C).
\(^b\) Number of sample units in count range.
\(^c\) Values expressed as fg ATP/CFU ± SD.
\(^d\) ---, not applicable.
TABLE 3. Relative quenching of bioluminescence by ground beef samples based upon internal ATP standardization.

<table>
<thead>
<tr>
<th>Range of ( \log_{10} ) CFU/g</th>
<th>Single processor</th>
<th>Retail samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( n^a )</td>
<td>ATP/RLU ± SD</td>
</tr>
<tr>
<td>4.5-6.0</td>
<td>12</td>
<td>56.5 ± 15.7</td>
</tr>
<tr>
<td>6.0-7.0</td>
<td>6</td>
<td>35.4 ± 5.6</td>
</tr>
<tr>
<td>7.0-8.0</td>
<td>3</td>
<td>27.5 ± 4.0</td>
</tr>
<tr>
<td>&gt;8.0</td>
<td>3</td>
<td>30.4 ± 3.1</td>
</tr>
<tr>
<td>Samples diluted 1:10d</td>
<td>24</td>
<td>20.6 ± 1.9</td>
</tr>
<tr>
<td>Reagent blanks</td>
<td>16</td>
<td>18.0 ± 2.1</td>
</tr>
</tbody>
</table>

\(^a\)Numbers of samples in given count range or sample category.
\(^b\)Values expressed as mean fg ATP/RLU ± SD.
\(^c\)--, not applicable.
\(^d\)Sample preparation diluted 1:10 for bioluminescent assay after NRS/Somase treatment.

TABLE 4. Percentages of residual nonmicrobial and reagent blank RLU in ground beef samples after treatment with NRS/Somase.

<table>
<thead>
<tr>
<th>Range of ( \log_{10} ) CFU/g</th>
<th>Single processor</th>
<th>Retail sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nonmicrobial(^b)</td>
<td>Reagent blank(^b)</td>
</tr>
<tr>
<td>4.5-5.0</td>
<td>50.6-86.0</td>
<td>2.6-13.5</td>
</tr>
<tr>
<td>5.0-6.0</td>
<td>24.5-67.2</td>
<td>1.8-19.5</td>
</tr>
<tr>
<td>6.0-7.0</td>
<td>16.8-31.4</td>
<td>1.1-3.1</td>
</tr>
<tr>
<td>7.0-8.0</td>
<td>4.5-8.5</td>
<td>0.4-0.9</td>
</tr>
<tr>
<td>&gt;8.0</td>
<td>0.3-2.0</td>
<td>0.1-2.0</td>
</tr>
</tbody>
</table>

\(^a\)Values represent residual nonmicrobial RLU in sample portion (NRS) + total RLU in sample portion (NRB) × 100%.
\(^b\)Values represent RLU in reagent blank (NRB, corrected for quenching) + total RLU in sample portion × 100%.
\(^c\)--, not applicable.

calculated for each sample using internal standardization and is summarized according to ranges of APC (20°C) in Table 3. For single source samples, mean ATP/RLU values of samples with APC’s ≤ \( \log_{10} \) 6.0 were nearly twofold higher than those of samples (undiluted for luminescence assay) with APC’s > \( \log_{10} \) 6.0. There was little variation in ATP/RLU values for diluted samples, with values being similar to those of reagent blanks. Decreases in ATP/RLU values were associated with increasing storage time and APC levels of samples during the first 6 d of storage and thus may be related to concomitant oxidation of meat pigments as well as changes in biochemical composition of samples as a function of time and microbial activity. This trend is also illustrated in Figure 2 by comparing ATP and RLU curves throughout storage. Mean ATP/RLU values for retail samples were similar to those in corresponding single source samples but exhibited a greater degree of variation which may be a reflection of greater variation in sample composition. Although ATP/RLU values of retail samples varied approximately threefold, correlations for RLU values were only slightly less than those for ATP values (Table 1).

The relative contribution of luminescence by nonmicrobial sources, i.e., residual nonmicrobial or somatic ATP (NRS measurement) and reagent blanks, is summarized in Table 4. It should be noted that these contributions were subtracted from the total luminescence (NRB measurement) to obtain net microbial ATP or RLU values for preceding comparisons. As expected, percentage contribution of nonmicrobial luminescence were greater at lower levels of APC (20°C), whereas contributions for single source samples having APC (20°C) ≥ \( \log_{10} \) 7.0 were less than 9 and 1% for residual, nonmicrobial ATP and reagent blanks, respectively. Much greater variation was observed among retail samples for residual, nonmicrobial ATP contributions. These results indicate: (a) that the NRS/Somase treatment to eliminate nonmicrobial ATP is not completely effective and (b) the advantage of measuring residual nonmicrobial ATP (NRS) along with total ATP for quantification of net microbial ATP, particularly at low APC levels. For example, the correlation between gross ATP measurements (NRB) and APC (20°C) was 0.82 as compared to 0.86 between net microbial ATP (NRB-NRS) and APC (20°C) for retail samples. Correlations with total ATP measurements were equivalent to those with net microbial ATP measurements for single source samples, probably as a result of low variation in sample composition and/or somatic ATP content. Although percentage contributions of residual nonmicrobial ATP decreased throughout storage of single source samples, RLU values for residual nonmicrobial ATP increased from approximately \( \log_{10} \) 2.0 to \( \log_{10} \) 3.0 RLU. Since ATP in microbial or somatic cells is assumed to be rapidly degraded upon death of the cell, these results
suggest an accumulation of free ATP in samples during storage or some degree of ATP extraction from old or debilitated bacteria whose cell envelopes could be less resistant to the NRS reagent.

The sensitivity of the Lumac bioluminescent ATP assay system is purported by the manufacturer to be ca. 10³ bacteria/ml under ideal conditions but ca. 10⁴ bacteria/ml in foods considering various interfering factors described previously. Thus, a sensitivity of ca. 10³ bacteria/g of ground beef would be expected in a sample homogenate diluted 1:10. This was, in fact, the lower range of sensitivity observed in this study as evidenced by the presented data in Tables 3 and 4 and Figure 4. While this degree of sensitivity limits the application of this bioluminescent method to many food systems, it is adequate for microbial assessments of most fresh meats, including ground beef. It is also interesting to note that the percent standard deviation (% SD) between triplicate portions of retail sample homogenates ranged from 0.3 to 11.3%, with an average of 3.5% for bioluminescent values, whereas corresponding % SD for APC (20°C) portions ranged from 0.3 to 34.4%, with an average of 14.5%. Stannard and Wood (23) found that error and sensitivity associated with ATP measurement were not significantly different (P>0.05) than those associated with plate counts of meat samples.

In summary, high overall correlation and linearity between bioluminescent measurements and APC (20°C) or APC (7°C) were obtained for 75 ground beef samples over a range of log₁₀ 4.8 to 9.9 CFU/g for APC (20°C). Corresponding correlations for APC (35°C) were less than those for APC (20°C or 7°C) but relatively high (r = 0.94) for single source samples. Despite variation in ATP/CFU values and quenching observed among many samples, RLU measurements were essentially as effective as ATP values in estimating APC’s. Correlations between bioluminescent and APC measurements were much higher for ground beef samples obtained from a single processing operation than for those from various retail markets due to greater homogeneity among single source samples. These results indicate that bioluminescent methods are applicable to rapid estimation of microbial levels in ground beef, particularly in individual processing operations, and offer great potential for prompt assessment of the microbial condition of raw materials or products throughout processing operations for various meat products.

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