Use of a Titrimetric Method to Assess the Bacterial Spoilage of Fresh Beef

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A new method of determining bacterial spoilage in fresh beef is presented. The technique is based on the fact that as beef undergoes refrigerator spoilage, there is a gradual increase in the production of alkaline substances by the spoilage flora. The level of these substances was measured by titrating meat homogenates to a pH 5.00 end point, employing 0.02 N HCl and an autotitrator. When 23 samples of ground beef from retail stores were tested, an average of 1.32 ml of acid was required for titration of 1 g of fresh beef to pH 5.00, whereas 2.58 ml was required for the same meat at the onset of spoilage. Preliminary data indicate that beef which requires more than 2 ml of 0.02 N HCl/g to lower its pH to 5.00 under the conditions of the test is in some state of incipient spoilage. The statistical correlation between titration values, log bacterial numbers, and extract-release volume was high ($P < 0.001$). The technique is simple to execute and is highly reproducible, and duplicate samples can be run within 15 min.

Although the degree of freshness or spoilage of meats is often evaluated by plate counts, it is known that spoilage is not the result of bacterial numbers per se but is caused by biochemical changes brought about by the growing flora. Investigations on the mechanism of spoilage of fresh refrigerated meats over the past several years have led to the proposal of a number of techniques for assessing its presence and extent. Among these are techniques based upon the phenomena of extract-release volume (ERV), water-holding capacity (WHC), meat swelling, and viscosity (1, 2, 5, 6). All four of these are based primarily on changes in hydration capacity of meat proteins, which is lowest for fresh meat but gradually increases as spoilage occurs.

Although the pH of fresh beef is around 5.6 to 5.8 and gradually increases to as much as 8.5 when beef becomes putrid, the increase from freshness to incipient spoilage generally does not exceed 0.3 to 0.5 of a pH unit. This, along with the fact that the change is usually not uniformly distributed in a meat sample, makes direct $p$H measurements unsuitable for the purpose of detecting incipient spoilage. Also, beef is often judged as spoiled without any noticeable $p$H changes.

The present report describes a more direct technique for detecting incipient beef spoilage by accurate titration of the basic (alkaline) substances or functions produced in beef by the spoilage flora. The technique employs the measurement of the quantity of dilute acid required to bring beef homogenates to pH 5.00. The volumes of acid have been correlated with log bacterial numbers, ERV, and pH on beef from different sources and in different stages of spoilage.

**MATERIALS AND METHODS**

Titrations were carried out by blending 10-g samples of beef in 100 ml of deionized water for 2 min and filtering through cheesecloth to eliminate connective tissue. Duplicate samples of the homogenate containing 2 g of meat each were titrated with 0.02 N HCl by using an autotitrator (model TTT, and ABU1, Radiometer, Copenhagen). The amount of acid required to bring the homogenates to pH 5.00 was recorded. The initial pH of homogenates was read simultaneously on the titrator.

The relationships between titration and spoilage, aging, and fat content were studied employing semitendinous (ST) muscle. To study the effect of spoilage on titration values, 15-g samples of ground muscle were stored at 5 C in small beakers covered with aluminum foil. Log bacterial numbers, $p$H, and titration values were determined on the stored meat at 2-day intervals. For the study of aging, 15-g samples of meat were stored at 5 C in gas-impermeable plastic bags as previously described (3). The effect of fat on titration values was determined by adding

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known quantities of beef fat to fat-free ST ground muscle followed by immediate titration. ERV values, log bacterial numbers, and percentage of fat were determined as previously described [1].

RESULTS AND DISCUSSION

The relationship between log bacterial numbers, pH, and titration values in fresh ground ST muscle undergoing spoilage at 5 C is presented in Fig. 1. During spoilage, log bacterial numbers, titration values, and pH showed a marked increase from the 2nd day of storage to the 10th; spoilage was detected on the 5th day, at which point the log bacterial count was 9.1/g, the titration volume was 2.9 ml, and the pH was 6.3. Beef stored in plastic bags, however, showed only a slight increase in both pH and titration values after 10 days of storage when the log bacterial count did not exceed 8.90/g. This meat was judged acceptable, even at the end of this 10-day holding period of 5 C.

With respect to titration volume of ground beef, the method of sampling may affect results. Since ground beef spoilage at refrigerator temperatures is largely due to surface growth, surface samples would yield higher titration volumes than those taken from the interior. To minimize this difference, the entire batch of ground beef [1 to 1.5 lb (453 to 679 g) portions] was thoroughly mixed by use of spatula, followed by the removal of test samples in a random manner. Replicate samples from ground beef treated in this manner gave results with low degrees of variation. When beef cuts are to be tested, both surface and interior portions should be mixed as for ground beef. The effect of mixing surface samples with interior samples, where there are generally fewer bacteria, is to dilute the generally higher level of titration substances present in surface samples and to neutralize any microbially produced organic acids that may be present in subsurface portions.

A pattern similar to that presented in Fig. 1 is presented in Fig. 2, employing retail-store stew beef which was ground in the laboratory. Statistically, the correlation coefficients (r) between titration values and pH and between titration values and log bacterial numbers were significantly above the 1% level; however, between titration values and ERV, r was significantly above the 2% level.
The effect of fat content on titration is presented in Table 1, from which it can be seen that mean values for four replicates decreased from 1.73 ml to 0.91 ml as the percentage of fat increased to 50. It was concluded from these findings that fat alone does not contribute to the acid-titratable groups in fresh beef, although there is some evidence that fat may affect titratable functions as beef undergoes spoilage.

**Table 1. Effect of fat content on titration values of fresh beef**

| Percent fat | Replicates | Mean | Standard deviation |
|-------------|------------|------|--------------------|
|             | 1         | 2    | 3     | 4    | 5    |
| 0           | 1.64      | 1.67 | 1.75 | 1.86 | 1.73 | 0.084 |
| 10          | 1.56      | 1.62 | 1.96 | 1.93 | 1.76 | 0.177 |
| 20          | 1.95      | 1.85 | 1.59 | 1.43 | 1.70 | 0.207 |
| 30          | 1.20      | 1.28 | 1.34 | 1.20 | 1.25 | 0.058 |
| 40          | 1.15      | 1.20 | 1.33 | 1.23 | 1.23 | 0.065 |
| 50          | 0.89      | 0.88 | 0.92 | 0.94 | 0.91 | 0.076 |

In an effort to determine the performance of titration on market meats, hamburger meat was obtained from 23 retail chain stores, and titration values, log bacterial numbers, ERV, pH, and fat content are presented in Table 2. Titration values, log bacterial numbers, ERV, and pH are given at freshness (day of purchase) and at onset of spoilage. The titration volumes of fresh hamburger meats ranged from 0.53 to 2.15 with a mean value of 1.32 ± 0.36, whereas values at the onset of spoilage ranged from 1.34 to 3.41 with a mean of 2.58 ± 0.56. The average time for the onset of spoilage was 5 days, with a range of 2 to 7 days at 5 C. With respect to its degree of sensitivity to the changes that occur when fresh beef undergoes spoilage, the titration volume increased by 95.4% from freshness to spoilage, based on mean values. The per cent increase with respect to bacterial numbers was 24.8, with the mean log number for fresh beef being 7.41 ± 0.61 and 9.25 ± 0.56 at the first signs of detectable spoilage. With respect to ERV, the per cent increase was 44.1; the mean at freshness was 34, whereas the mean value at spoilage decreased.

**Table 2. Titration values, log bacterial numbers, extract-release volume (ERV), pH, and fat content of hamburger meats from 23 stores at freshness and at onset of organoleptic spoilage**

| Store no. | Titration value | Log no. | ERV | pH | Per cent | Days to Spoilage |
|-----------|----------------|---------|-----|----|----------|------------------|
|           | Fresh Spoilage onset | Fresh Spoilage onset | Fresh Spoilage onset | Fresh Spoilage onset | Fresh Spoilage onset | Fresh Spoilage onset | Fresh Spoilage onset | Fresh Spoilage onset | Fresh Spoilage onset |
| 1         | 0.92 1.70        | 7.51    | 8.63 | 34 23 | 5.9 6.3 | 16.5 5       |
| 2         | 0.53 3.40        | 7.88    | 9.41 | 41 17 | 5.6 7.2 | 31.5 5      |
| 3         | 0.81 2.70        | 6.40    | 9.00 | 37 19 | 5.9 6.9 | 39.0 7      |
| 4         | 0.92 2.38        | 7.20    | 8.26 | 35 27 | 5.9 6.3 | 26.5 7      |
| 5         | 1.20 1.56        | 7.18    | 8.94 | 31 24 | 6.0 6.1 | 25.6 3      |
| 6         | 1.27 1.34        | 6.75    | 8.80 | 32 27 | 6.0 6.1 | 22.5 3      |
| 7         | 1.25 3.36        | 7.23    | 10.08| 33 11 | 6.1 7.0 | 21.0 5      |
| 8         | 1.35 2.17        | 7.18    | 9.71 | 36 14 | 6.1 7.0 | 36.0 5      |
| 9         | 1.25 2.03        | 7.52    | 9.00 | 34 25 | 5.9 6.3 | 27.0 6      |
| 10        | 1.33 2.77        | 7.62    | 9.24 | 30 20 | 5.8 6.5 | 24.0 6      |
| 11        | 1.70 2.87        | 7.95    | 9.58 | 26 17 | 6.3 6.7 | 16.8 3      |
| 12        | 1.31 2.65        | 6.52    | 9.82 | 40 19 | 6.0 6.5 | 33.0 7      |
| 13        | 2.15 2.34        | 7.91    | 9.30 | 25 18 | 6.3 6.6 | 25.0 2      |
| 14        | 1.43 2.94        | 7.11    | 9.45 | 41 28 | 6.0 6.3 | 26.6 5      |
| 15        | 1.39 2.52        | 8.82    | 8.99 | 43 23 | 5.9 6.4 | 28.0 6      |
| 16        | 1.50 2.30        | 6.90    | 8.68 | 36 21 | 5.9 6.3 | 22.0 6      |
| 17        | 1.52 3.15        | 7.60    | 9.52 | 37 12 | 6.0 6.7 | 31.4 5      |
| 18        | 1.26 3.15        | 7.38    | 9.61 | 36 12 | 5.9 6.8 | 16.5 4      |
| 19        | 1.35 2.51        | 7.52    | 8.38 | 34 22 | 6.0 6.5 | 30.0 5      |
| 20        | 1.53 2.42        | 7.41    | 9.08 | 30 20 | 6.1 6.5 | 36.0 4      |
| 21        | 2.05 3.41        | 8.32    | 9.81 | 29 10 | 6.2 6.9 | 40.0 4      |
| 22        | 1.05 2.53        | 7.17    | 9.45 | 34 17 | 5.9 6.8 | 27.5 5      |
| 23        | 1.37 3.15        | 7.48    | 10.00| 36 10 | 6.0 6.9 | 30.5 5      |
| Mean      | 1.32 2.58        | 7.41    | 9.25 | 34 19 | 5.99 6.59 | 27.5 5  |
| SD*       | 0.36 0.56        | 0.60    | 0.56 | 6.6 | 5.3 0.32 | 0.33 6.6 |

* Standard deviation.
TABLE 3. Titration values of hamburger samples related to log bacterial numbers of 8.50/g and to extract-release volume (ERV) of 25

| Store no. | Titration values | At log 8.50/g | At ERV of 25 |
|-----------|------------------|--------------|-------------|
| 1         |                  | 1.65         | 1.68        |
| 2         |                  | 1.60         | 2.45        |
| 3         |                  | 2.30         | 2.28        |
| 4         |                  | 2.53         | 2.45        |
| 5         |                  | 1.48         | 1.50        |
| 6         |                  | 1.33         | 1.38        |
| 7         |                  | 2.20         | 2.00        |
| 8         |                  | 1.80         | 1.75        |
| 9         |                  | 1.60         | 2.00        |
| 10        |                  | 1.65         | 2.15        |
| 11        |                  | 2.10         | 1.83        |
| 12        |                  | 2.13         | 2.25        |
| 13        |                  | 2.23         | 2.15        |
| 14        |                  | 2.33         | 3.15        |
| 15        |                  |              |             |
| 16        |                  | 2.20         | 2.08        |
| 17        |                  | 2.30         | 2.30        |
| 18        |                  | 2.20         | 2.13        |
| 19        |                  | 2.68         | 2.20        |
| 20        |                  | 2.10         | 2.00        |
| 21        |                  | 2.18         | 2.33        |
| 22        |                  | 1.61         | 1.85        |
| 23        |                  | 2.10         | 2.13        |

Mean | 2.01 | 2.10 |
SD<sup>b</sup> | 0.37 | 0.35 |

<sup>a</sup> No data.
<sup>b</sup> Standard deviation.

log bacterial count per gram of 7.91, but an ERV of 25 at freshness; whereas sample 21 had a titration value of 2.05, a log bacterial count of 8.32, and an ERV of 29 at freshness. Both of these samples of meat were apparently undergoing incipient spoilage at the time of purchase.

A simplified technique for the rapid detection of spoilage in ground beef can be achieved by adding 2 ml of 0.02 N HCl/g of meat to the blended and filtered homogenate and checking the final pH of the homogenate. Using this method, when the pH is >5.0, the meat may be presumed to be in some state of incipient spoilage.

Although the identification of the basic substances that are titrated by this technique is not known at this time, all available evidence suggests that they are microbially produced and their appearance is time-dependent. When fresh beef was inoculated with meat spoilage flora to log 8.5 to 9.0/g and tested immediately, titration values remained low and no signs of spoilage resulted. The homogenizing step, along with the constant stirring that accompanied titration, suggests that the substances in question are mainly nonvolatile. In a previous report from this laboratory (4), amino sugar complexes were shown to increase along with bacterial numbers and hydration capacity. The possibility exists that these compounds are at least partly responsible for the increased amounts of acid necessary to lower the pH of spoiling beef. Further research towards identification of the basic functions and their role in meat spoilage is in progress.

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