Evaluation of Rapid Tests for Monitoring Alterations in Meat Quality During Storage

I. Intact Meat

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ABSTRACT

Seven rapid analytical tests (color value, thiobarbituric acid number, extract release volume, pH, "tyrosine" value, pH_t, and redox potential) were evaluated as possible indicators of bacterial contamination in intact meat. Color value is a reflectance value related to hedonic acceptance of the meat. Comparisons of results from these seven tests with determination of bacterial load (plate count) and with time of storage were analyzed statistically to determine the relative contributions of bacterial action and of intrinsic reactions. The color values and "tyrosine" values were the most effect monitors of bacterial contamination. Although the thiobarbituric acid test effectively monitored changes in meat quality, it was not specific for those produced by bacteria. The remaining tests were ineffective under conditions employed.

Proteolysis and lipid oxidation occur in fresh meat during storage. Detectable unfavorable organoleptic changes appear when the number of bacteria exceed $10^6/cm^2$ for intact meat (*8*). Bacteria count is generally a reliable criterion of spoilage. The determination of bacterial levels, however, requires a minimum of 48 h of incubation for an accurate count, while physical and chemical changes caused by high bacterial populations and storage conditions can be measured more rapidly and could frequently be a convenient alternative.

The tests selected for evaluation had to meet the following criteria: they must be rapid with results available within 1 h, they must be accurate with a small (< 50 g) sample of meat, and they must be easy to do with commonly available lab equipment. The seven analytical tests chosen, color value, thiobarbituric acid number, extract release volume, pH, "tyrosine" value, pH_t, and redox potential meet these criteria.

Surface color alteration is the most obvious change during storage. Strange et al. (*11*), using a reflectance spectrophotometric method to follow surface color changes, found a high correlation of reflectance data with consumer acceptability.

Meat pH changes with the increasing bacterial population. Shelef and Jay (*10*) reported that the pH of beef rose with increasing bacterial growth, and described a rapid method to detect spoilage involving measurement of pH following addition of a standard volume of HCl to a filtered homogenate of the meat.

Pearson (*6*) reported that EMF (electromotive force) of spoiled meat may fall below –250 mv. Initially, EMF of fresh high quality meat is high; the effect of this high EMF on microbial growth is to prolong the initial lag phase in the growth curve (*4*).

Another rapid method purported to predict microbial quality of beef is extract release volume (ERV) (*2*). ERV is related to water holding capacity of meat (*3*) which is highly correlated with pH (*12*).

Pearson (*7*) demonstrated that the "tyrosine" value of meat increased with storage time along with total volatile nitrogen until amino acid deamination by the aerobic metabolism of pseudomonads limited formation of free amino acids. He indicated the "tyrosine" value also measured other reductants soluble in trichloroacetic acid such as tryptophan, cysteine, phenolics, sulfhydryls etc.

A deteriorative change not necessarily caused by microbial contamination is lipid oxidation. The thiobarbituric acid (TBA) test measures the carbonyl residues resulting from lipid peroxidation and the method for TBA analysis used was a variation of the procedure devised by Witte et al. (*13*).

The rate of the above changes depends on initial bacterial load, physical and biochemical state, availability of oxygen, temperature of storage, and muscle composition.

We evaluated the selected tests for effectiveness in monitoring microbial quality. Since intrinsic changes occur in meat during storage in addition to changes caused by bacterial action, experiments were designed so that effects of bacterial action alone could be identified.

The various tests have previously been used in studies of storage change in ground meat, not necessarily in

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relation to bacterial contamination and generally have not been evaluated for use on intact meat. Our studies show that their values on ground and intact meat are strikingly different. The present report concerns applications to intact meat.

EXPERIMENTAL

Materials

Three sides of beef (graded USDA Good) were obtained within 90 min post slaughter. Samples of longissimus dorsi (l.d.) muscle (ca. 100 g) were removed for immediate analysis. The rest of the l.d. was removed and cut into samples the next day. Samples used for color evaluation and bacteria counts were cut across the grain into approximately 50-g portions each with a surface area of approximately 60 cm^2. The remaining l.d. was divided into 35-g samples. Each sample was wrapped in oxygen-permeable meat wrap (PVC stretch film MC-FMC) and randomly assigned to storage at −1 or 7 C.

Methods

All tests were done on samples stored at −1 C and 7 C to obtain insight as to the relative effects of the intrinsic changes in meat and the effects of bacterial growth on meat. An ideal storage temperature for fresh meat is −1 C. At this temperature (−1 C) meat is not frozen but the rate of bacterial growth is greatly retarded. At 7 C, bacterial growth is accelerated. Thus the choice of these temperatures allows differentiation of the effects of intrinsic changes from the effects of bacterial growth.

Bacteria plate counts. Meat samples for bacteria counts were shaken with a sterile 0.1% peptone solution contained in sterile quart Mason jars. Appropriate dilutions were made before spreading on nutrient agar and incubating for 3 days at 20 C. All dilutions were plated in triplicate. The bacteria counts are reported as $\log_{10}$ of the actual count.

Color. The color value, % reflectance at 630 nm minus % reflectance at 580 nm or Δ%R, of the meat was determined according to the method of Strange et al. (*11*) using a Beckman[2] DBG recording spectrophotometer equipped with a diffuse reflectance attachment.

Extract release volume. Fifteen grams of meat were blended with 60 ml of water for 2 min and filtered immediately through a Whatman # 1 filter (15 cm in diameter) folded in the manner described by Jay (*3*). Volume of filtrate, termed ERV, was measured after 15 min of filtration.

pH, pH_t, EMF. The pH of the ERV filtrate was measured with a combination probe glass electrode. The EMF was measured on the same ERV filtrate using a platinum electrode and a silver-silver chloride half-cell. The platinum electrode was standardized with a ferrous-ferric standard EMF solution (*5*). The pH_t, adapted from the method of Shelef and Jay (*10*), was determined by adding 2 ml of 0.05 N HCl solutions to a 10-ml aliquot of the ERV filtrate and noting the changed pH. This new pH was called pH_t.

TCA extract. Twenty grams of meat were blended with 50 ml of cold 20% trichloroacetic acid (TCA) for 2 min. The blender contents were rinsed with 50 ml of water, mixed together, and filtered through a Whatman #1 filter. This filtrate is termed the TCA extract and is used in the TBA and tyrosine tests.

TBA number. The TBA number was determined using a variation of the method described by Witte et al. (*13*). A 5-ml aliquot of the TCA extract was mixed with 5 ml of 0.01 M 2-thiobarbituric acid. Either of two procedures was used for TBA color development. One procedure involved storage for 14 h at room temperature (ca. 20 C) and the other for 30 min at 100 C. Color development, measured as Absorbance (A) at 532 nm, was identical when either color development procedure was used with standard solutions of tetraethoxypropane or with TCA extracts of meat. Absorbance at 532 nm is reported as the TBA number.

[2] *Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.*

Tyrosine value. Two and one half ml of the TCA extract were diluted with 2.5 ml of water. To this 10 ml of 0.5 N NaOH were added followed by 3 ml of Folin's Reagent (diluted 1 Folin's:2 water). After mixing, the color was developed for 15 min at room temperature before reading at 660 nm. The "tyrosine" value is reported as mg of tyrosine/g of meat (*7*).

RESULTS AND DISCUSSION

The intact samples of l.d. were stored and analyzed for bacteria counts and the seven physical and chemical changes as described in the experimental section. Bacterial contamination as well as intrinsic changes in meat during storage are causes for the changes measured in the quality tests selected for evaluation.

Data in Fig. 1 indicate that the bacterial population grew more slowly on meat stored at −1 C than at 7 C. Throughout most of the storage period (20 days) there were at least 3 to 4 log differences in bacterial numbers between the two temperatures.

Δ%R and "tyrosine" value versus time for meat stored at both temperatures are shown in Fig. 2. Δ%R decreased more rapidly after 5 days storage and "tyrosine" value increased more rapidly for meat stored at 7 C.

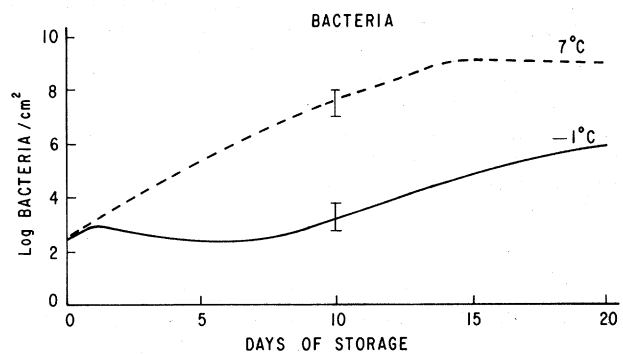

Figure 1. *Bacteria, - - - - Longissimus dorsi stored at 7 C; — Longissimus dorsi stored at —1 C. Vertical brackets in this and in successive figures represent range of determinations for the day indicated.*

Figure 3 shows the TBA number and the pH_t number versus day of storage. The TBA numbers increase during storage but no definite differences were observed between meats stored at the two temperatures.

The pH_t for the meat stored at 7 C increased more than the pH_t for the meat stored at −1 C. This increase occurred near the end of the storage period. However, the size of this increase was smaller than the variations in pH_t among carcasses.

ERV and pH versus time are shown in Fig. 4. ERV increased rapidly and pH decreased rapidly during the first few hours after slaughter. These changes were expected due to onset of rigor. During storage the ERV for meat held at both temperatures decreased slowly but variations among duplicates were larger than the decrease noted. After the rapid initial decrease, pH stayed at approximately the same level until, at extremely high levels of bacterial contamination ($>10^8/cm^2$), it rapidly increased about 0.4 pH unit. EMF values versus

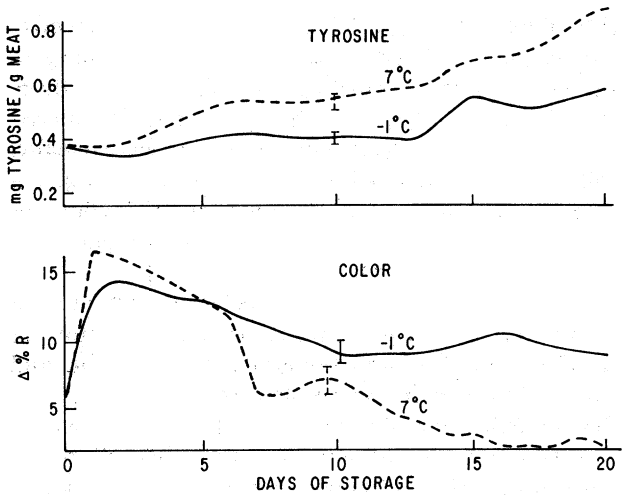

Figure 2. *"Tyrosine" value, - - - - Longissimus dorsi stored at 7 C; — Longissmus dorsi stored at -1 C. Color (Δ %R), - - - - Longissimus dorsi stored at 7 C; — Longissimus dorsi stored at -1 C.*

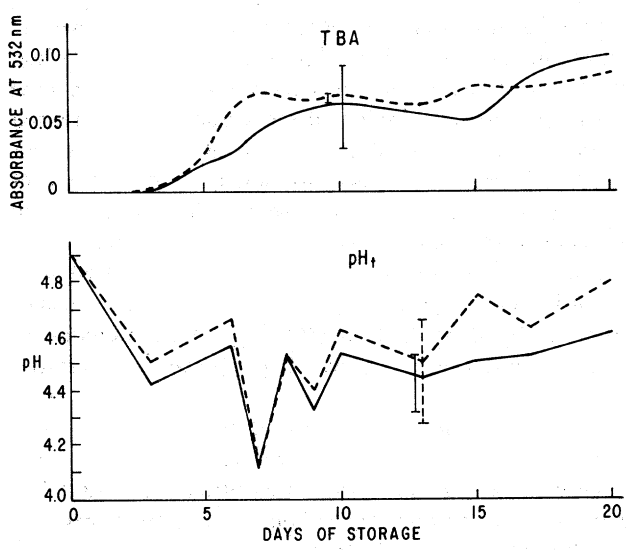

Figure 3. *Thiobarbituric Acid Number, - - - - Longissimus dorsi stored at 7 C; — Longissimus dorsi stored at -1 C. pH_t - - - - Longissimus stored at 7 C; — Longissimus dorsi stored at -1 C.*

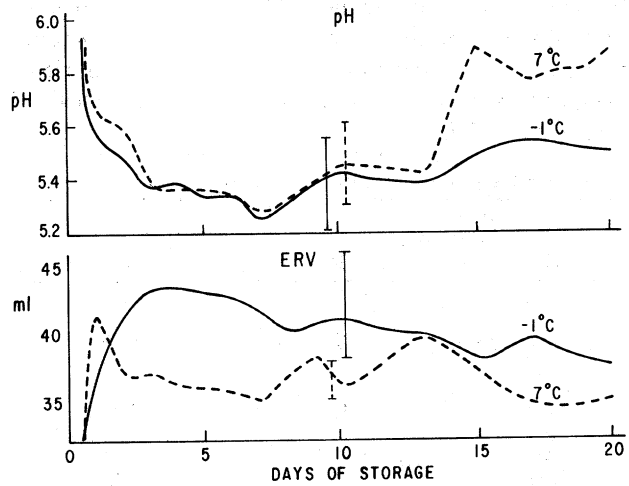

Figure 4. *pH, - - - - Longissimus dorsi stored at 7 C; — Longissimus dorsi stored at -1 C. Extract Release Volume, - - - - Longissimus dorsi stored at 7 C; — Longissimus stored at -1 C.*

time are not shown. These values were so erratic that any trends are meaningless.

Data from meat stored at both temperatures were pooled and statistical analyses carried out on the pooled data for each quality test. Table 1 lists the linear correlation coefficients ("r" values) for the $\log_{10}$ bacteria counts/cm² versus the seven quality tests. Correlation with Δ%R, "tyrosine" value, TBA number, and pH_t were significant at the 99% confidence level.

"Tyrosine" value, TBA number, pH and pH_t may be expected to increase as bacteria increase while Δ%R, ERV, and EMF may be expected to decrease according to the literature previously cited. Our results agree with the expected direction of the change.

Intrinsic changes in meat were assumed to occur as the meat aged during storage and would be related to the length of storage. Table 2 shows the "r" values for storage time in days versus eight quality test results, determination of $\log_{10}$ bacteria count/cm² being the eighth test. Bacteria count, Δ%R, "tyrosine" value, TBA number, and EMF all had significant, "r" values, the directions of the changes being as predicted in the literature previously cited.

TABLE 1. *Correlation of quality tests with log bacteria/cm²*

Quality test	Linear correlation coefficient	Number of samples
Color (Δ %R)	–753[a]	103
"Tyrosine" value	.696[a]	72
TBA number	.491[a]	76
Extract release volume	–.223	65
pH	.266	65
pH_t	.348[a]	64
EMF	–.364	29

[a]P > 99%.

TABLE 2. *Correlation of quality tests with storage time in days.*

Quality test	Linear correlation coeffieicent	Number of samples
Log bacteria counts/cm²	.678[a]	103
Color (Δ%R)	–.512[a]	103
"Tyrosine" value	.528[a]	72
TBA number	.507[a]	76
Extract release volume	–.060	94
pH	.261	94
pH_t	.209	64
EMF	–.497[a]	29

[a]P > 99%.

Δ%R, "tyrosine" value, and TBA number were the only quality tests which correlated closely with both bacteria count and time of storage. Table 3 gives the probabilities that the "r" values for the quality tests versus bacteria count and versus length of storage differ. Δ%R was more highly correlated with bacteria count than with length of storage. "Tyrosine" value was also more highly correlated with bacteria count than with length of

TABLE 3. *Comparison of quality test for predicting log bacteria count/cm², storage time, and color.*

Quality test	Linear correlation coefficient			P[a]
	Bacteria count	Storage time	Color	
Color (Δ%R)	−.75	-.51	—	.002
"Tyrosine" value	.69	.53	—	.14
TBA number	.49	.51	—	.88
TBA number	.49	—	-.53	.70
TBA number	—	.51	−.53	.82

[a]Probability the r's are equal.

storage but not to the same extent at Δ%R. This observation is not obvious if Fig. 2 alone is used for evaluation of the quality tests.

TBA number versus bacteria count, versus length of storage, and versus Δ%R all have about the same "r" values. The "r" value for TBA number versus color was calculated. Benedict et al. (*1*) reported that lipid oxidation in ground meat can have a negative effect on color. The effects of length of storage, bacteria counts, and color changes on TBA numbers cannot be separated with these data.

As a further check on the validity of the quality tests as indicators of meat quality the pooled data were divided into two classes: quality test data from meat samples with low bacteria counts $\leqslant 10^4/cm^2$ and quality test data from meat samples with high bacteria counts $\geqslant 10^7/cm^2$. Quality test data from meat samples with bacteria counts $> 10^4/cm^2$ and $< 10^7/cm^2$ were not used. The Student's "t" test was used to test the hypothesis that the quality test results on low bacteria count meat are not different from the results of quality tests on high bacteria count meat. Δ %R, "tyrosine" value, and TBA number all had "t" values that did not support this hypothesis. Δ %R's for low bacteria count meat were significantly higher than the Δ %R's for high bacterial count meat. "Tyrosine" values and TBA numbers for low bacteria count meat were significantly lower than for high bacteria count meat (Table 4).

Of the seven quality tests evaluated, Δ%R is the most effective monitor of meat quality during storage. Δ%R is a measure of the degree of meat pigment oxidation. Several precautions should be observed when using this quality test. Meat with insufficient time to bloom fully will give a low Δ %R even though of good quality. Bloom is the conversion of myoglobin (a purple meat pigment) to oxymyoglobin (a red meat pigment) by oxygen. Concentration of myoglobin in the muscle also affects Δ%R. The muscle type and the age of the slaughtered animal will affect myoglobin levels (*9*). Occasionally, meat with an extremely high bacteria count will give a higher Δ%R than expected due to reduction of met-myglobin (a brown meat pigmeat) to myoglobin by the reducing capacity of bacteria present.

"Tyrosine" value was also an effective monitor of meat quality. The "tyrosine" value is an indicator of proteolysis as it measures the amino acids tyrosine and tryptophan present in a nonprotein extract of meat.

TBA number gave significant results in these tests but its correlations with bacteria and with time of storage were less than those of either Δ%R or "tyrosine" value. The TBA number may be a better quality monitor with a meat that is more easily oxidized than intact beef, such as ground beef or pork.

ERV did not predict or monitor meat quality as well as expected, but it had a significant "r" value when compared with pH. The pH of a water extract of the meat was not a sensitive monitor of meat quality. It increased when the number of organisms exceeded 10^8 but not reliably. However, pH measurements on the surface of the meat may be a more effective monitor because with intact meat most alterations occur on the surface.

pH_t had a significant "r" value when compared with bacteria count and it followed the same trends predicted by Shelef and Jay (*10*), but it did not change in intact meat to the extent that they reported with ground meat.

EMF of meat has promise as an effective quality test. However, methodology must be worked out to reduce the large variation in the EMF's measured. We did EMF measurements on only a few of our meat samples because variability was extremely high.

In conclusion, Δ%R was the most effective monitor of bacterial contamination in intact meat. It is nondestructive and convenient to use. The next most effective monitor was "tyrosine" value. As a monitor for bacterial quality, it was effective but interference due to intrinsic changes in meat was more likely to affect the "tyrosine" value than Δ %R.

TABLE 4. *Comparison of mean quality test data on high bacterial count ($\geqslant 10^7$ / cm²) and low bacteria count ($\leqslant 10^4$ / cm²) meat.*

Quality test	Low bacteria count meat		High bacteria count meat		t[b]
	Mean	N[c]	Mean	N[c]	
Color (Δ%R)	13.23	45	6.79	31	5.37[a]
"Tyrosine" value	.46 mg/g meat	27	.73 mg/g meat	28	4.67[a]
TBA number	.05 A	30	.16 A	29	4.50[a]
Extract release volume	39.6 ml	27	37.0 ml	16	2.38
pH	5.35	26	5.53	22	2.31
pH_t	4.38	26	4.48	22	1.47
EMF	−154 mv	13	−209 mv	7	1.58

[a]Quality test means for low and high bacteria count meat were different. P < 99.9%.
[b]Student's t value.
[c]Number of samples.

TBA number increase was correlated about equally with bacteria numbers and with storage time. However, changes in the TBA number were small and usefulness of TBA as a monitor for bacterial quality in intact meat appears limited.

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