Relationship Between Lactic Acid Concentration and Bacterial Spoilage in Ground Beef

PATRICIA S. NASSOS,* A. DOUGLAS KING, JR., AND ALLAN E. STAFFORD

Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Berkeley, California 94710

Lactic acid concentration correlated with organoleptic spoilage of refrigerated, coarsely ground beef stored in casings with low oxygen permeability. The samples were assayed over time for lactic acid concentration, total aerobic plate count, percentage of gram-positive organisms, and pH. Lactic acid increased in all samples, as did the bacterial counts and percentage of gram-positive organisms in the total microflora, the latter representing an increase in the lactic acid-producing bacteria. pH was found to decrease in all samples, with the smallest decrease in pH being observed in the meat sample which maintained the lowest proportion of gram-positive organisms. With samples evaluated by a sensory panel, lactic acid levels were found to correlate inversely with odor acceptability.

Storage of refrigerated ground beef in oxygen-impermeable casings is used extensively to retard spoilage (15, 19, 22). The anaerobic environment which ensues from this type of packaging encourages the growth of non-proteolytic lactic acid-producing microorganisms which appear to compete successfully with the aerobic proteolytic flora usually associated with obvious spoilage (6, 20). Consequently, the storage life of refrigerated beef can be increased to several weeks or even months (4, 22).

With prolonged storage, however, unfavorable organoleptic changes will eventually occur in refrigerated ground beef packaged in wrap with low oxygen permeability. The lactic acid-producing microflora, although not proteolytic, are associated with the development of a sour odor, which causes beef to be considered unacceptable by consumers (9, 20). Sutherland et al. make a distinction in type of spoilage by referring to proteolytic spoilage as "sweet-rotten spoilage" and that caused by lactic acid producers as "acid/sour spoilage" (25). In meat wrapped in casings with low oxygen permeability, we can assume that both forms of spoilage will occur as long as both types of microflora are present. The degree to which proteolytic bacteria contribute to the spoilage is dependent on the number which remain viable in the meat product, which in turn is dependent on the oxygen permeability of the storage wrap.

Bacterial counts are generally thought to be an indicator of early spoilage, with "off" odors becoming apparent when bacterial numbers reach approximately 10^7 cells per g of meat (2, 25). Unfortunately, bacterial counts are time consuming, taking from 5 to 10 days for accurate assessment of psychrotrophic bacteria. There is also some disagreement as to whether total bacterial counts correlate with organoleptic appraisal of the meat product and whether counts can be used to assess future shelf life (12, 16, 20, 26). Proteolytic bacteria, such as some *Pseudomonas* spp., will cause spoilage at lower numbers than lactic acid-producing organisms.

Several tests other than total bacterial counts have been proposed to measure the microbial quality of processed meat. These tests, which include indicator dye methods, extract release volume, pH, and titratable acidity, have proven to be of limited value and are not commonly used. Reductase tests, using certain dyes which act as hydrogen acceptors in measurement of dehydrogenase levels, have been tried for determining spoilage in beef (11). These methods are not feasible for use with ground or minced beef products due to release of cellular reductones during the grinding process.

Jay (13, 14) found a correlation between the volume of aqueous extracts released by beef homogenate and the microbial quality of the meat. The phenomenon, which he termed extract release volume, appears to be similar in some respects to the water-holding capacity of meat. Unfortunately, the method allows appraisal of good meat and samples with obvious spoilage with a broad area of uncertainty between. Also, different muscle tissue from the same

animal can give different results. Extract release volume appears to be caused by changes in meat protein from bacterial proteolysis or autolytic processes and may not be an indicator of incipient spoilage by non-proteolytic organisms such as lactic acid producers. Other methods which use pH or titrimetric determinations of meat homogenates show changes with spoilage, but differences between a fresh and obviously spoiled meat product are not great enough for practical use (15, 25).

Spoilage does not appear to be the result of bacterial numbers per se, but is caused by biochemical changes which occur in the course of microbial growth. Sharpe has suggested that measurement of a metabolic by-product may give a better indication of food quality than actual numbers of organisms present (24). Bacterial counts do not indicate whether the microflora present are innocuous or promoting spoilage. We investigated the possibility of correlating a bacterial metabolic product with early spoilage. Since the predominant microflora found in ground beef packed in a relatively oxygen-impermeable casing produce lactic acid as a major metabolic by-product, we felt there may be a correlation between this particular organic acid and early spoilage.

MATERIALS AND METHODS

Sample preparation. Eight coarsely ground beef samples (½-in. [1.27 cm] diameter die cut) weighing approximately 20 lb (ca. 9.07 kg) each were procured over a period of 3 weeks from a local distributor within 24 h of grinding and packaging. The beef samples were packaged in casings with relatively low oxygen permeability (40 ml of O_2 m^{-2} day^{-1} atm^{-1} at 22.8°C) and consisted of three different grades rated by fat content; two samples were very lean (12 to 14%), three were lean (15 to 19%), and three had medium fat content (22 to 25%). Portions were removed from each sample initially within 2 h of procurement for analysis of lactic acid, aerobic plate count of bacteria present, determination of the ratio of gram-positive to gram-negative organisms, pH determination, and organoleptic panel appraisal. Approximately 2 lb of meat from one end of the pack was removed. This meat, suspected of being exposed to oxygen from the previous pack opening, was discarded. A second segment of meat (approximately 1 lb) was then removed from the casing and divided into appropriate size portions for the analyses. The remainder of the sample was secured in its casing for future analyses at 3- and 4-day intervals for a total of six sampling periods. The beef was stored at 7°C throughout the study.

Bacteriology. Bacterial counts were done on 50-g samples aseptically removed from the casing and homogenized with sterile 0.1% peptone water for 2 min at high speed in a Waring blender. Appropriate serial dilutions with 0.1% peptone water were surface plated in duplicate on plate count agar (Difco Laboratories, Detroit, Mich.). Inoculated media were incubated aerobically at 20°C for 5 days. At the end of the

incubation period, colonies were enumerated from duplicate countable plates (50 to 300 colonies per plate). Fifty colonies were randomly selected from the agar plates and Gram stained. The ratio of gram-positive to -negative colonies was calculated for each of the samples.

Chemical analysis. Samples were prepared for lactic acid analysis by homogenizing approximately 400 g of coarsely ground beef for 1 min in a Cuisinart food processor (model DLC-10) with a metal chopping disk. The resulting beef had the appearance of thick homogeneous paste. A 25-g subsample was added to 215 ml of 0.2 N HCl and 10 ml of an internal standard solution containing 72 mg of glutaric acid. This mixture was homogenized, centrifuged, and filtered, and 10 ml of the filtrate was lyophilized according to the procedure of Harvey et al. (10) for nonvolatile water-soluble organic acids. After lyophilization, the sample was esterified with boron trifluoride (15% [wt/vol]) in propanol (BF$_3$-propanol) according to the procedure of Salwin and Bond (23), except the amounts of BF$_3$-propanol and saturated $(NH_4)_2SO_4$ used in the assay were increased to 10 ml, and CHCl$_3$ was increased to 5 ml.

The resulting propyl derivatives of lactic and glutaric acid were measured with a Hewlett-Packard gas chromatograph (model 5720A) equipped with a flame ionization detector. The glass column was packed with 80/100 mesh Chromosorb W.-H.P. coated with 10% AT-1000 (Altech Associates, Deerfield, Ill.). Helium flow at the detector was 30 ml/min, and the detector and injector were operated at 300 and 225°C, respectively. The column was programmed from 100 to 180°C at 8°C/min. So as not to interfere with subsequent analysis, higher-molecular-weight fatty acid esters were removed from the column by programming to 240°C and holding this temperature for 6 min. The peaks of interest were measured quantitatively with a Hewlett-Packard 3390A integrator.

Fat content was determined by the Foss-let method described in *Official Methods of Analysis* (1). An Orion Research Digital Ionalyzer (model 701A) was used for pH measurements. A filtrate of a 1:10 (meat sample to distilled water) homogenate was used for the pH determinations.

Sensory appraisal. The three coarsely ground lean beef samples with lean-grade fat content were rated by a 20-member sensory panel on both odor and appearance. Sample rating was determined with a standard nine-point hedonic scale (a score of 9, like extremely; a score of 1, dislike extremely). Panel members were also asked to note whether the meat sample was acceptable or unacceptable in both odor and appearance. Each sample was appraised twice within a 6-h period, within 2 h after removal from its package. Approximately 50 g of sample being judged for appearance was placed in a glass petri dish and viewed under daylight fluorescent light against a neutral gray background. Panel members were given a background explanation of the product they were appraising, insofar as a coarsely ground beef sample shows more actual fat and connective tissue than would be noticeable once the meat is reground. For odor evaluation, a portion of the meat sample was presented in glass-stoppered 125-ml Erlenmeyer flasks wrapped in tissue to mask appearance. The odor samples were judged in air-conditioned booths under 7.5-W green bulbs.
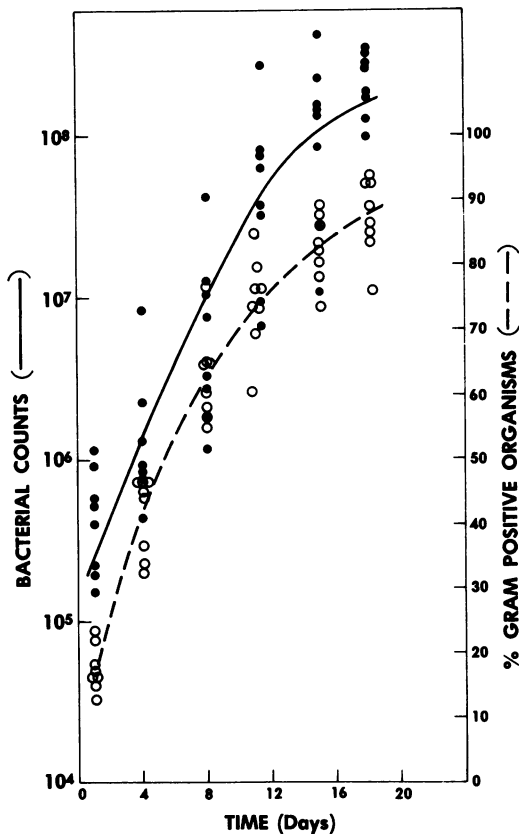
FIG. 1. Comparison of total aerobic bacterial counts and the shift to predominance of gram-positive microflora with time. Symbols: ●, bacterial counts; ○, percent gram-positive organisms.

RESULTS AND DISCUSSION

Total aerobic bacterial counts increased with time in all eight of the meat samples (Fig. 1). Initial counts ranged from 10^5 to 10^6 bacterial cells per g of wet weight of beef. At the end of 18 days, bacterial counts had reached 9×10^7 to 3×10^8 bacterial cells per g in all meat samples. Variations in counts between samples appeared to be random with regard to fat content of the meat. All samples showed an increase in the proportion of gram-positive to -negative organisms over time as determined by analysis of 50 randomly selected colonies. This was in agreement with a previous study conducted in our laboratory (results not shown) in which we identified microflora in 1- to 3-week-old ground beef samples stored at 7°C. Identification of isolates was accomplished with the aid of *Bergey's Manual of Determinative Bacteriology* (3). There was a marked shift with age of meat from gram-negative flora, identified as *Pseudomonas* spp., to gram-positive organisms. We noted that

greater than 95% of the gram-positive organisms were bacteria which characteristically produce lactic acid. Gram-positive flora were found to be predominantly *Leuconostoc* and *Lactobacillus* spp., with some *Streptococcus* spp. A very small percentage of gram-positive organisms were of the *Micrococcus* spp. Because of these findings, the Gram stain was used in the present study to observe changes in the proportion of lactic acid-producing microorganisms over time.

Our beef samples initially contained 12 to 23% gram-positive organisms (Fig. 1). By day 18, the percentage of gram-positive organisms amounted to 75 to 94% of the total colonies assayed. In addition to reducing oxygen concentration, the lactic acid flora appear to inhibit growth of proteolytic gram-negative spoilage flora such as *Pseudomonas* species (5, 8, 18). Pierson et al. have found lactic acid-producing bacteria to approach 100% of the total microflora present in ground beef samples stored in vacuum-wrapped, oxygen-impermeable casings (20). In the present study, meat samples were not vacuum packaged, although casings were relatively impermeable to oxygen. Because of this, strict aerobic organisms could continue to grow, particularly near the surface of the meat pack, and it is unclear whether the proteolytic gram-negative microflora would have been more completely replaced by gram-positive organisms had the study continued.

pH values of all meat samples ranged from 5.6 to 5.9 initially (Table 1) and showed slight decreases, giving final values between 5.0 and 5.8 after 18 days. Samples with medium-grade fat content generally showed higher pH readings compared to meat samples with very lean or lean grade content. Seven samples showed a slight but steady pH decline, giving final pH readings more than 0.3 pH unit below the initial readings. The remaining sample had a pH decline on day 4, but an increase on day 11, giving a final pH 0.1 unit below the initial reading. This same sample

TABLE 1. Change in pH over time for ground beef samples with different fat contents

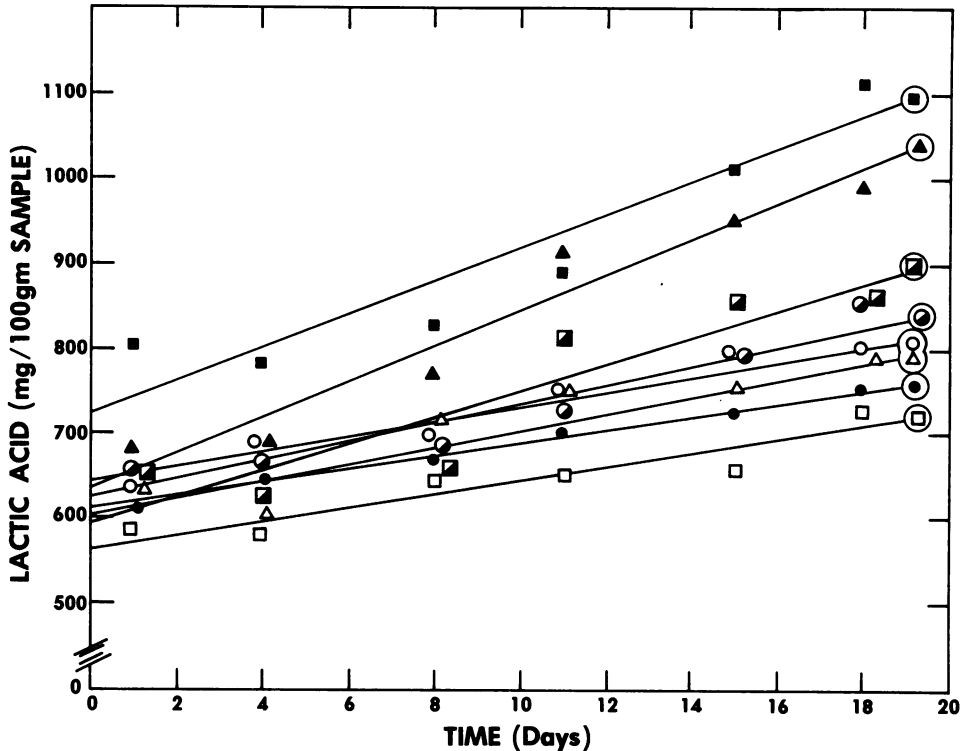| Sample	pH on day:					
	1	4	8	11	15	18
Very lean	5.6	5.6	5.3	5.3	5.1	5.0
(12 to 14% fat)	5.7	5.5	5.3	5.2	5.1	5.2
Lean	5.7	5.7	5.5	5.4	5.4	5.3
(15 to 19% fat)	5.7	5.6	5.5	5.4	5.4	5.4
	5.8	5.8	5.5	5.3	5.3	5.2
Medium	5.8	5.8	5.6	5.5	5.5	5.5
(22 to 25% fat)	5.9	5.7	5.7	5.8	5.8	5.8
	5.9	5.6	5.4	5.4	5.5	5.5

FIG. 2. Change in lactic acid concentration over time in individual meat samples. The same symbols indicate data points for a particular meat sample. Lines represent best fit of the data points.

had the lowest proportion of gram-positive organisms for the last two testing periods compared with the other seven samples. These findings are in agreement with the idea that low pH measurements are associated with growth of lactic acid-producing microflora, which are favored by the low-oxygen environment. The decrease in pH was not enough, however, to predict meat spoilage.

Lactic acid values were adjusted for fat content of the beef, as there is not an appreciable amount of lactic acid in fat but there is an endogenous level found in muscle tissue. The lactic acid concentrations increased during the study (Fig. 2). Samples with initially higher lactic acid concentrations had higher final concentrations. Differences in initial lactic acid levels can be explained, to a small extent, by the different mammalian lactic acid concentrations at the time of slaughter; more stressed animals would have lower muscle lactic acid concentrations (21). After death, an aseptic conversion of muscle glycogen to lactic acid occurs, unless glycogen stores have been exhausted in the stressed animal. Stress to animals before slaughter is avoided because of the undesirable dark, firm, and dry condition of the meat associated with muscular activity (17). Therefore, the dif-

ferences in initial lactic acid concentration attributable to glycogen conversion are probably slight. A larger contribution to differences in initial lactic acid concentration may be due to differences in microbial populations and bacterial production of lactic acid. Sizeable variations were noted in initial bacterial counts as well as variations in the percentage of lactic acid-producing organisms present.

Lactic acid levels increased with bacterial numbers (Fig. 3). The correlation between lactic acid concentration and bacterial count was statistically significant at $P < 0.0001$. The Kendall Tau B correlation coefficient was 0.66, reflecting the wide range of bacterial counts seen at lactic acid levels around 800 mg/100 g of sample. Viable bacterial counts peaked at approximately 10^8 cells per g, whereas lactic acid values continued to increase, showing continued production of this metabolic product by the microflora. A continued increase in lactic acid after maximum population size was reached may have been due, in part, to the shift in the proportion of proteolytic to lactic acid-producing microflora. Over time, proteolytic microflora made up a smaller proportion of the total bacterial count. Some proteolytic bacteria are known to utilize lactic acid (7) and would have caused a decrease in
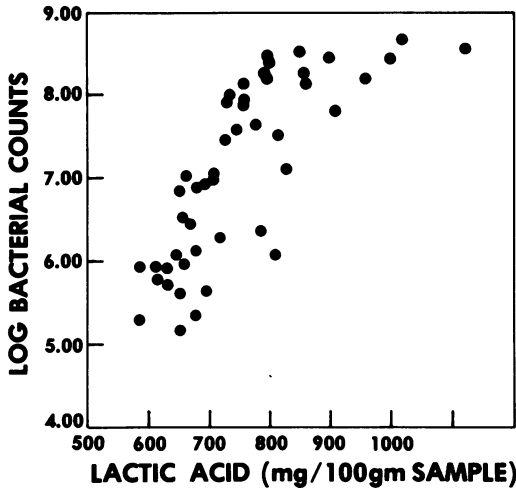
FIG. 3. Relationship between total aerobic bacterial counts and lactic acid concentration of the meat samples.

lactic acid concentration earlier in the study when present in greater numbers.

Sensory panel evaluation of the meat samples showed hedonic score and percent acceptability for appraisal of both odor and appearance to be statistically significant ($P < 0.0001$), with Kendall coefficients of 0.78 and 0.71, respectively. Appraisal of meat odor seemed to have greater indicative value for early spoilage than did meat

appearance (Table 2). Average odor scores ranged from 2.7 to 5.5 and showed a more consistent decrease with age of meat than did appearance scores. Odor acceptability ranged from 10 to 81%. When panelists were asked to appraise meat appearance, the hedonic scores and percent acceptability showed a smaller decrease than that seen in odor appraisal, with values ranging from 6.4 to 4.2 for appearance score and 93 to 50% for acceptability. Panelists judging appearance had difficulty in appraising the ground beef in its coarsely ground form. Therefore, we instructed them to disregard large fat particles and visible connective tissue which were invariably present.

We found a correlation between lactic acid levels, which ranged from 627 to 857 mg/100 g of meat, and odor acceptability (Fig. 4). The correlation was statistically significant at $P < 0.05$, although the Kendall correlation coefficient was small ($r = 0.47$), due mainly to the large variation in odor acceptability with lactic acid values less than 700 mg/100 g of meat. Below this concentration, odor acceptability decreased from 81 to 24% as the lactic acid level increased. All samples having lactic acid values greater than 704 mg/100 g of meat rated less than 50% acceptability by our panelists. An upper 90% confidence interval for 50% odor acceptability was calculated as a lactic acid concentration of 725 mg/100 g of meat. Samples containing lactic acid concentrations greater than 725 mg/100 g of meat would likely be found unacceptable by

TABLE 2. Lactic acid concentration and organoleptic panel appraisal of ground beef with lean grade (15 to 19%) fat content

Day of study[a]	Lactic acid (mg/100 g of meat)	Odor[b]		Appearance[b]	
		Score[c]	% Acceptability	Score[c]	% Acceptability
1	649	4.9	78	4.7	63
	655	4.5	53	4.8	65
	650	5.5	81	6.4	93
4	690	5.0	74	4.9	67
	674	3.7	40	5.4	83
	627	5.2	78	5.9	88
8	704	3.6	35	4.5	60
	676	3.1	24	4.9	69
	652	3.9	38	4.9	70
11	753	3.1	35	4.7	78
	727	2.9	20	4.7	53
	809	3.6	38	5.1	70
15	795	3.2	25	4.2	61
	792	3.3	28	4.8	58
	857	3.7	30	4.8	80
18	788	2.7	10	4.5	50
	846	3.2	28	4.7	65
	852	3.8	25	5.3	80

[a] Three separately procured samples were appraised on each day.
[b] Values are the average of two appraisals conducted within a 6-h period.
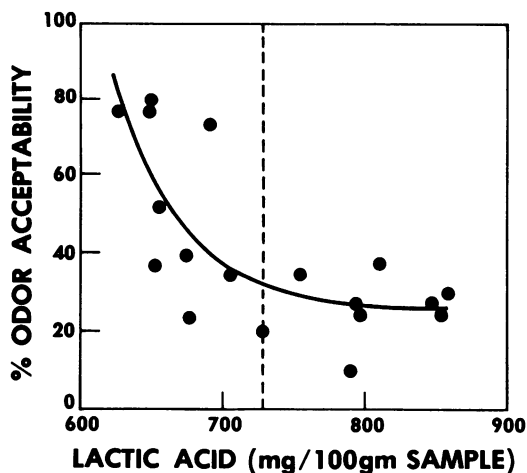[c] Rating score from a standard nine-point hedonic scale.

FIG. 4. Relationship between percent odor acceptability and lactic acid concentration in lean beef samples (15 to 19% fat content). Solid line is a fitted asymptotic regression line. Dashed line indicates the upper 90% confidence limit on 50% odor acceptability.

odor evaluation at least 50% of the time. Meat samples with lower lactic acid values would need further evaluation to determine acceptability.

We conclude that lactic acid determinations are useful as a screening assay to select meat samples which would be considered undesirable by half of the population doing the evaluation. This selection process would be of value for agencies and institutions which are responsible for the procurement of large quantities of ground beef and must make decisions regarding acceptance or rejection in a relatively short time. The example above shows an upper limit for acceptability set at a concentration where half of the population would find the product unacceptable. A different level of acceptability could be set depending on the needs of the particular evaluating group. This method of early spoilage detection has an advantage over other methods previously described in that a measurement of lactic acid does not merely appraise proteolytic spoilage, but rather off-odor associated with meat packaged in relatively oxygen-impermeable casings, a packaging method in which ground beef is routinely stored and shipped.

Sensory panel evaluations were conducted only on beef samples with lean grade fat content. It is reasonable to assume that samples with lower and higher fat content would exhibit similar changes in odor and appearance. The lactic acid concentration corresponding to acceptance 50% of the time on the basis of odor may differ, however, with large differences in fat content.

Findings from this study can be used, as shown in the example, to establish criteria for accepting or rejecting meat for purchase based upon a rather rapid chemical analysis. Further studies are needed to determine how lactic acid values in the coarsely ground beef correlate with spoilage once meat has been reground, as a high degree of aeration will cause a population change from the non-proteolytic lactic acid producers to proteolytic *Pseudomonas* spp. Evidence by Gill points to lactic acid being utilized by proteolytic microflora which are responsible for frank spoilage in aerobically stored meat (7). Higher lactic acid levels may, in part, cause their rapid growth and subsequent proteolytic activity in the reground beef.

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