COMPARISON OF THE RATES OF pH REDUCTION AND LACTIC ACID ACCUMULATION DURING GLUCOSE FERMENTATION IN BEEF SAUSAGE

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SUMMARY: Fermentation measures of pH reduction (hydrogen ion concentration) and lactic acid accumulation (g/100 g sausage) at 24.3 to 45.0°C were generally found to fit first order reaction rates for fermentation by Pediococcus acidilactici, Pediococcus pentosaceus and Lactobacillus plantarum during their active growth phase. Activation energies for glucose conversion to lactic acid were 2.4 to 4.3 times higher when determined from pH reduction as compared to determinations based on lactic acid production. Results of the study indicated that the buffering capacity of muscle proteins and other sarcoplasmic substances for hydrogen ions is an important variable in measuring the extent of fermentation through pH reduction.

INTRODUCTION: Many processors of fermented sausages monitor the development of acidity in a fermenting meat mix by following pH decline to a target of pH 5.3 or less (Bacus, 1984). Fermentation temperatures utilized are those recommended as "optimum" for the specific lactic acid bacteria present in the sausage mix. The "Good Manufacturing Practices" (GMPs) for fermented sausage (AMI, 1982) include as a critical control point, a time-temperature relationship which should be voluntarily met in achieving a pH of 5.3 or less. The GMPs also imply that risk reduction, to assure safe and wholesome products in the marketplace, can be attained by the addition of lactic acid bacterial starters for fermentation.

General chemical kinetics also involves time-temperature relationships to express the rate of a reaction. Potentially, kinetic analyses can be applied to the bacterial conversion of glucose to lactic acid if viewed as an "active reaction" proceeding at a given "rate" for a known temperature. The bacterial fermentation of glucose in sausage can be monitored by measuring the rate of pH decline or the rate of lactic acid accumulation. However, the amount of pH reduction in relation to the actual acidity produced in muscle tissue systems is significantly affected by the presence of inherent buffering activity within the tissue (Swatland, 1984; Bacus, 1984). The objective of this study was to determine the kinetics of the overall fermentation of glucose to lactic acid in a beef sausage system containing various lactic acid bacteria. The lactic acid bacteria selected for this study were Pediococcus acidilactici, P. pentosaceus and Lactobacillus plantarum.

MATERIALS AND METHODS: Sausage mixes consisting of an initial 5.7 kg of ground beef were prepared with the following quantities of additional ingredients (per kg of meat): 0.156 g $NaNO_2$, 0.47 g sodium erythorbate, 30.0 g NaCl, 7.5 g seasoning mix, 10.0 g glucose, 50.0 ml water and 5.05 ml of a starter culture suspension of P. acidilactici or P. pentosaceus (LACTACEL® 110, LACTACEL® 74, respectively, Microlife Technics, Inc., Sarasota, FL) or L. plantarum (ABC18, ABC Research Laboratories, Gainesville, FL). Each culture addition yielded approximately 2 x 10^7 cells/g sausage mix. The sausage mixes were stuffed in 32 mm diameter cellulosic casings and formed into links of approximately 100 g each. After initial sampling of the links (0 hr), sets of the links were fermented and sampled at the following temperature-time periods: 24.3 and 32.0°C for 4, 8, 10, 12 and 14 hr; and 37.3, 40.0 and 45.0°C for 2, 4, 6, 8 and 10 hr. At each time interval, duplicate sausages were slurried, the pH was measured and the slurry was then titrated to an endpoint of pH 8.30. The pH values were converted to hydrogen ion concentration ($[H^+]$) and the meq of NaOH in titration (total acidity) were converted to g lactic acid/100 g sausage sample.

All kinetic analyses for the starter cultures were conducted using data of the active fermentation phase, i.e., time periods during maximum change in total acidity content or $[H^+]$. First order reaction kinetics were used to analyze the conversion of glucose to lactic acid and its subsequent effect on pH through H^+ generation. First order reaction rate constants (k's) were determined using the following equation (Kittsley, 1963): $k = 2.303/t[\log (y_{initial}/y_{final})]$, where t is the time interval of the active phase of fermentation (hr) and y is the initial and final total acidity or $[H^+]$ in the time interval. From analysis of variance by the general linear model using slope analysis and the lack of fit test (SAS, 1985), the Arrhenius relationship of: $\log k = b_0 + b_1(1/T)$, where k is the rate constant (hr^{-1}), b_1 is the slope, T is expressed in °K and b_0 is the intercept, was examined for acid development and H^+ accumulation. The activation energies (Ea) for lactic acid and H^+ production were calculated using the slope relationship to Ea (Kittsley, 1963).

Three replicate processing and fermentation trials were conducted for each of the three lactic acid bacteria studied. The data were analyzed using general linear model analysis of variance (SAS, 1985).

RESULTS AND DISCUSSION: The lengths of the lag and "active fermentation" phases were separated by applying first order reaction kinetics to the concentration increases in total acidity and H^+ concentration. The assumption for a first order kinetic fit was based on the fact that bacterial growth, at appropriate temperatures, usually fits first order rates in the exponential growth

phase (Lamanna et al., 1973). Linear regression models in the "best linear fit" of maximum total acidity and maximum H^+ changes over all cultures and temperatures showed the following: (1) correlation coefficients (r) of 0.84 or higher and 9 of 13 model slope coefficients (b_1) for total acidity development were significant at $p<0.10$; and (2) for H^+ production, 10 of 13 model slope coefficients (b_1) were significant at $p<0.10$ and the correlation coefficients were 0.85 or higher. The approximate length (hr) of the lag phase and the interval (hr) of the active fermentation phase at each temperature during fermentation of beef sausage by each of the three lactic acid bacteria are given in Table 1. No data are given for L. plantarum at 40.0 and 45.0°C due to sample spoilage prior to slight acid production.

First order reaction rate constants for total acidity development and H^+ production were determined and are given in Table 2. The rate constants decreased with increasing temperature. The negative k values resulted from utilizing the increase of end product concentrations (acidity or H^+) for the kinetic evaluations rather than the decrease in reactant concentration (glucose). The coefficients of determination (R^2) from general linear model regression analysis for the Arrhenius relationship, using the first order rate constants for total acidity development and H^+ production by cultures of P. acidilactici and P. pentosaceus (Table 2), were significant ($p<0.05$ to $p<0.01$). The significance levels in the Arrhenius relationship when L. plantarum was used was $p<0.24$ (total acidity) and $p<0.16$ (H^+); however, lack of fit analyses in all cases indicated that linear fits were the appropriate models for the respective rate constants. The temperature dependency of the rate constants was used to determine the Ea's required for the fermentation of glucose (Table 3). Since the kinetic analyses were conducted on the basis of products generated, negative Ea values were obtained; the values should be viewed as positive energy inputs of the same kcal/mole for the overall glucose fermentation process.

The Ea's for lactic acid production from fermentation of glucose in beef sausages by P. acidilactici, P. pentosaceus and L. plantarum were 2.90, 3.37 and 4.43 kcal/mole respectively (Table 3). The Embden-Meyerhof-Parnas glycolytic pathway used by homofermentative bacteria for metabolic conversion of glucose to lactic acid (Jay, 1986) has many intermediate steps. These Ea's represent the overall net energy required as an input for the reaction pathway used by each of the lactic acid bacteria in the respective temperature zones that were studied. When the Ea's were based on the kinetics of H^+ production from pH reduction, the net energy requirements were 2.4 (L. plantarum) to 3.8 (P. acidilactici) to 4.3 (P. pentosaceus) times higher than when based on lactic acid accumulation. The Ea's for H^+ production by P. acidilactici, P. pentosaceus and L. plantarum were 9.51, 14.55 and 10.73 kcal/mole, respectively.

Fermentation analyses by the two measures of total acidity and pH reduction confirm that direct measurement of acidity from bacterial fermentation by means of pH values is subject to buffering effects of the meat tissue and/or other ingredients used in processing. Swatland (1984) indicated that meat contains buffering substances such as proteins, dipeptides (carnosine and anserine) and ATP which can capture H^+. Thus, the buffering activity of the beef sausage system acted as an additional energy barrier, increasing Ea's when glucose fermentation by the lactic acid bacteria was kinetically based on pH measurements.

CONCLUSIONS: Kinetic analyses were successfully applied to the fermentation of glucose in beef sausage by cultures of P. acidilactici, P. pentosaceus and L. plantarum. The results suggest that meat fermentations should be targeted at attaining specific acidity concentrations during fermentation rather than the current industry practice based on specific pH endpoints.

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Table 1. The approximate length (hr) of the lag phase and the intervals (hr) of the active fermentation phase as based on maximum rates of acid production and hydrogen ion (H^+) concentration increase during fermentation of beef sausage by three lactic acid bacteria.

Temperature °C	P. acidilactici				P. pentosaceus				L. plantarum[1]			
	Total Acidity[2]		$[H^+]$		Total Acidity		$[H^+]$		Total Acidity		$[H^+]$	
	Lag	Active	Lag	Active	Lag	Active	Lag	Active	Lag	Active	Lag	Active
24.3	8	8-14	8	8-14	8	8-14	10	10-14	8	8-14	8	8-14
32.0	4	4-12	4	4-12	4	4-14	4	4-14	8	8-12	8	8-14
37.3	2	2-8	2	2-8	2	2-8	4	4-10	6	6-10	6	6-10
40.0	2	2-8	2	2-10	2	2-8	4	4-10	-	-	-	-
45.0	2	2-8	2	2-8	2	2-8	4	4-8	-	-	-	-

[1] All sausage samples containing L. plantarum spoiled at 40.0 and 45.0°C prior to fermenting.
[2] Total acidity and hydrogen ion concentration ($[H^+]$) were examined in terms of g acid/100 g sausage and moles of H^+, respectively.

Table 2. First order rate constants for total acidity development and hydrogen ion production in the active fermentation phase for beef sausages containing a starter culture.

Temperature[1] (°C)	Total Acidity (1/hr x 10^{-1})			Hydrogen Ion (1/hr)		
	P. acidilactici	P. pentosaceus	L. plantarum	P. acidilactici	P. pentosaceus	L. plantarum
24.3	-0.3709	0.2206	0.4422	-0.07293	-0.04606	-0.11899
32.0	-0.5308	-0.6493	-1.0816	-0.18712	-0.34084	-0.30707
37.3	-0.7542	-0.7693	-1.1118	-0.26101	-0.51434	-0.35697
40.0	-0.8704	-0.8709		-0.31954	-0.57191	
45.0	-1.0666	-1.0533		-0.40686	-0.67939	
R^2	0.977 (p<0.01)	0.973 (p<0.01)	0.871 (p<0.24)	0.997 (p<0.01)	0.913 (p<0.05)	0.939 (p<0.16)

[1] R^2 from general linear model analysis of $\log k = b_0 + b_1(1/T)$, where k = rate constant, b_0 = intercept, T = absolute temperature (°K = °C + 273.18) and b_1 = slope.

Table 3. Energies of activation for total acid production and hydrogen ion increase during the active fermentation of glucose by three lactic acid bacteria in beef sausage.

Lactic Acid Bacteria	Temperature Range (°C)	Energy of Activation (kcal/mole)[1,2]	
		Total Acid Production	Hydrogen Ion Increase
P. acidilactici	24.3 - 45.0	-2.90 ± 0.26	-9.51 ± 0.29
P. pentosaceus	24.3 - 45.0	-3.37 ± 0.32	-14.55 ± 2.59
L. plantarum	24.3 - 37.3	-4.43 ± 1.70	-10.73 ± 2.74

[1] Mean ± SEM.
[2] Energy of activation is -4.576 b_1, where b_1 is the slope parameter from a general linear model regression analysis of $\log k = b_0 + b_1 (1/T)$. In the equation, k = the respective rate constant at a given T, T = temperature (°K) and b_0 = intercept.