Effects of processing parameters on certain microbiological and biochemical characteristics of fermented Italian dry salami manufactured under commercial conditions.

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Italian-style dry salami is a fermented meat product made of pork, beef, various spices and other non-meat ingredients and produced primarily on the West Coast of the United States at levels exceeding 150 tons per day. Most of the processing plants are in the San Francisco area where climatic conditions have been conducive for the natural aging of the product. Until recently most of the companies used either part of a previously fermented batch of salami as a natural lactic starter culture for fermentation of the next batch, or they used no added starter of any kind, relying on a lactic acid bacteria (LAB) present in the meat to ferment the salami mix. During the 70's, 5 outbreaks of staphylococcal food poisoning occurred from the consumption of fermented sausages. These events forced the industry to start changing the fermented sausage making process from an art to a science and develop good manufacturing guidelines for the production of safe products (1). Today most of the Italian dry salami manufacturers, including the 2 companies which cooperated in this project, use commercially available or customized lactic starters and fully controlled for temperature and relative humidity fermentation, ripening rooms. The purpose of this study was to quantify the effect of various processing parameters on certain microbiological and biochemical characteristics of dry salami produced under commercial conditions. A parallel study on the nature of the macrobiological changes during fermentation (3) led to the development of customized LAB starters.

MATERIALS AND METHODS

A. Sampling Procedure. Dry sausages were collected the 1st Monday of each month from 2 San Francisco area plants over as 45-month period. The diameters and weights of the sausages were 5.25 cm (8 oz.), 5.9 (18.5) and 7.7 (variable) for Company A and 5.3 cm (8 oz.), 6.35 (18.5) and 7.8 (variable for Company B. Production lost were sampled on day 0, 1, 3, 7, 14, 21 and 28 of processing. Individual sausages were shipped refrigerated to the laboratory in the afternoon and analyzed the next morning.

B. Sausage formulation and processing. The meat part of the sausage included pork shoulders (PS), pork jowls (PJ), pork fat (PF) and beef (B). The average % composition of meat ingredients for the small (S), medium (M) and large (L) size sausages of Company A was: S(PS 60.9, PJ 4.5, PF 10.2, B 24.4); M(PS 61, PJ 4.8, PF 9.6, B 24.7); L(PS 55.4, PJ 4.7, PF 5.3, B 31.2). The compositions for Company B was: S(PS 76.1, PJ 9.5, PF 0, B 14.4); M(PS 77.7, PJ 8.4, PF 0, B 13.9); L(PS 67.4, PJ 53, PF 2, B 25.3). The sausage meat fat content of both companies was 24%. The non-meat ingredients for Company A and B included NaCl (3.34, 3.21%), nonfat dry milk (3.14, 3.44%), glucose (3.14, 3.10%), spices (0.6, 0.92), nitrite (140, 140 ppm), nitrate (100, 100 ppm) for a total of 10.24, and 10.69%, respectively. Frozen pork was passed through a hydroflaker and then conveyed to a large mixer. Fresh ground beef and 0.01 to 0.015% ground 18-19 day sausage (natural starter) were added to the mixer containing the pork meat. A mixer full of sausage mix composed one lot. Amounts of 150-225 lb mix were transferred to a chopper for thorough mixing, addition of the non-meat ingredients and more mixing for about 1 minute. The mix was stuffed into fibrous casings. The sausages of Company B were next placed in the fermentation room at 16°C and 88 to 90% relative humidity (RH) for 24 hours and then at 24 to 26°C and RH 75 to 80% for two days. Ripening was completed at 15-18°C and RH 75 to 80% for 17-35 days, depending on sausage diameter. In Company A sausages were immediately placed into a fermentation room 23-25°C and RH 70-75% for 3 days, and then ripened at 18-19°C and 70-72% RH for 17-35 days.

C. Microbiological and Chemical Analyses. These analyses were done as described by Genigeorgis et al (6) in the companion paper. *Staphylococcus aureus* was identified and counted according to Metaxopoulos et al (11).

D. Statistical Methods. The biomedical computer programs, BMDP2V and BMDP1R (4), for analysis of covariance and multiple regression, respectively, were used statistical evaluation of the data. All variables were adjusted for the year of salami manufacture to remove the effect of year on the variables.

RESULTS

A. General. Mean values of 10 chemical and microbiological variables measured during the fermentation-ripening of 218 lots of salami produced by two companies are shown in Table 1. Analysis of covariance indicated no interaction between the responses for company and salami diameter for any of the variables at any process day. Thus, the difference in mean response observed for the two companies was consistent for the three diameter groups; the effect of company and diameter on the 10 response variables was additive. Satistically significant ($P<0.01$) company and/or salami diameter effects were observed for all variables except for *S. aureus* and LAB counts.

B. Effect of Company on Microbial and Chemical Changes. There were no significant differences in any of the geometric mean counts of *S. aureus* and LAB between Company A and B on any of the fermentation-ripening test days. The three highest *S. aureus* counts on each test day for each salami diameter of Company A and B are listed in Table 2. Five of the 218 lots tested (2/93 for Company A and 3/125 for Company B) had $>10^5$ *S. aureus*/g of salami on one or more test days. Only 10/1424 samples had *S. aureus* counts $\geq 10^5$/g. Table 3 shows the *S. aureus* LAB, pH and titratable acidity (TA) values of the high *S. aureus* count lots for day 0 through day 14. The mean test day responses for each lot are also indicated. The high *S. aureus* counts were observed on day 2, 3 and/or 7; all but one were >3 standard deviations (SD) from their mean. 3/5 lots had 0-day responses, differing significantly ($\geq$2 SDs) from their mean; both lots A-large (company and diameter, respectively) had low initial TA (2-2.1 SDs from mean); lot B-small had a low initial LAB count (>2 SDs from its mean) and low TA (2.2 SDs) on day 3 and 7; lot A-small had low LAB counts (2.3 SDs) on day 2. Salamis of Company B had significantly higher counts (0.5-1.4 $\log_{10}$ units/g more) of mannitol-fermenting, salt-tolerant (MS) organisms (mainly cocci) than salamis of Company A. Significant differences in the mean values of nitrite, color and water activity (a_w) between the salamis of Company A and B were observed mainly during the fermentation period (day 0 to day 3), while differencesin moisture, pH and TA values between companies were observed during the ripening period (day 3 to day 28). The only significant differences in brine values between the companies was on day 21.

C. Effect of Sausage Diameter on Microbial and Chemical Changes. There were no significant effects of diameter on the geometric mean counts of *S. aureus*, LAB and nitrite levels. Significant differences in MS counts among the diameters were observed only on day 14 and 21. The smaller the diameter, the greater the count. The level of nitrosomyoclobin (color) differed among the diameters on almost all test days with large diameter sausages

having more color. A_w differences occurred on day 2 through 14 with small diameter sausages having a lower a_w. pH and TA were significantly affected by salami diameter for day 7, 14 and 21, with the larger diameter salamis having the lowest pH and TA. Moisture and brine values differed among diameters on almost all test days with the larger diameter salamis having the most moisture and the least brine.

D. Effect of Day on Microbial and Chemical Changes. The geometric mean of S. aureus counts remained $<10^3$/g throughout the process period with a range $<10^2$-$1.9x10^5$ and $<10^2$-$1.1x10^6$ for Company A and B, respectively. The highest counts occurred between 2-4 days. The highest initial counts were $5.1x10^4$/g and $7.1x10^4$/g (means $1.4x10^2$ and $2.4x10^2$) for Company And and B, respectively. The geometric mean of LAB counts increased from $1.2x10^5$/g to a high of $2.3x10^8$/g during the fermentation and remained fairly leveled during ripening (day 3-28). The MS geometric mean counts increased from an initial level of about 10^4/g to a maximum of $4.7x10^5$-$3.7x10^6$/g per day and declined to 2.10^4-10^5/g at the end of the precess. The pH of the salamis decreased from 6.1 to 4.4-4.5 and paralleled the rise on TA. The latter reached a maximum of 1.3-1.5% in the finished product. The largest change in pH and TA occurred between day 0 and 2 following the significant increases of LAb counts. There was a precipitious decrease in the nitrite concentration of the salamis during the fermentation period, from the initial level of 140 ppm to approximately 20 ppm and to about 10 by the end of the ripening period. This decrease was paralleled by the increase in color level, especially during the fermentation period from about 12 to 70-80 ppm. During ripening color increased only slightly. The mean initial moisture of the salamis was 53-55% and in the finished product was 37 to 42%. The rate of moisture loss was directly related to the diameter of the salamis. A_w decreased with time to a final mean 0.89-0.905 depending on diameter. The % brine increased with time to >10% by day 14-21 depending on diameter.

E. Mulitple Regression Analysis. Evaluation of the data was attempted using multiple regression (4). High correlation (>0.7) between many of the variables caused the regression models to be biologically unexplainable. Path analysis also proved unsuccessful in developing any models. The simple correlations between some biologically associated variables were: LAB and pH, -0.6971; pH and nitrite, 0.8577; nitrite and color, -0.7510. Regression analysis of these relationships indicated that all were highly significant ($P<0.01$).

DISCUSSION

A. Microbial Changes. The fact that geometric mean counts of S. aureus did not exceed 10^3 per gram during the fermentation-ripening period can be attributed to (a) the prompt (competitive) growth and acidification of the salamis by LAB, (b) the relatively low fermentation temperature, and (c) the relatively low initial number of S. aureus in the salami mix (6). Repeated studies have shown that the growth of S. aureus and enterotoxin production in fermented sausages and model food systems is favored by the absence or low count of LAB (2, 12, 13), high initial S. aureus counts (11), higher than lower fermentation temperatures (7, 18), higher initial pH (12), and aerobic rather than anerobic conditions (2). From his studies of S. aureus in a number of foods, Tatini (20) concluded that oxygen tension and associative growth of other microorganisms affect S. aureus enterotoxin production more adversely than other factors such as temperature, pH and a_w. Metaxopoulos et al (11) found that the amount of growth of S. aureus was dependent on inoculum size when they inoculated salamis produced by Company B of this study, with 10^2 to 10^6/g levels of four strains of S. aureus. All strains grew at every level of inoculation. The investigators detected thermonuclease in salamis which were inoculated with $\geq10^4$ cells per gram, but only when growth reached levels $>10^7$ cells per gram. No enterotoxin was detected in any of the inoculated samples. They (12) further found that when they inoculated salamis with two levels of S. aureus (10^4 and 10^5/g) and varied the starter cultures (no starter, 10^5 and 10^6/g), the growth of S. aureus in the salamis was affected significantly ($P<0.005$) by the initial levels of S. aureus and LAB. The higher the initial level of S. aureus, the greater the growth of S. aureus, and the higher the LAB, the greater the inhibition of S. aureus. The high counts ($>10^5$/g) of S. aureus in five of the 218 lots of the present study (Table 3) appears to have been due to one or more of the following factors: (a) high initial S. aureus count in lot A-small, (b) low initial lactic count in lot A-large and B-small, (c) low initial TA in lot A-large and lot B-small, (d) slow growth of lactics in lot A-small, (e) slow acidity development in lot B-small, and/or (f) high fermentation temperature (27°C) in lot B-large. The latter lot was unusual in that it was held at 27°C during day 2 and day 3 of the fermentation rather than at 24 to 26°C. This higher-than-normal temperture may have resulted in the growth of more S. aureus. The other two diameters fermented with lot B-large (at 27°C) also had high S. aureus counts (4.61 and 4.04 log units) on day 2. The latter count were 2.0 and 2.2 SDs from their mean. Lot A-large was also unique, in that, in addition to having a low initial lactic count and a low initial TA, it was fermented without a starter. This would explain its low initial lactic count of 3.40 log units. No reason was found for the high S. aureus counts of lot B-medium.

Differences in product formulation may partly explain why the mean MS counts were significantly higher on day 0 (and thus remained higher during the fermentation-ripening period) in the salamis of Company B than of Company A. One formulation difference between companies was in the percent pork jowls added; the mean value for pork jowls in Company B's salami mix was 7.6%, whereas the mean value in Company A's mix was 4.6%. There are indications that some meats, such as pork jowls and cheeks, may be carrying unusually high numbers of staphylococci (8). However, in testing pork meat of salami mix, Santos and Razavilar (19) reported that there was no significant difference ($P<0.05$) in the number of samples of pork jowls and shoulders positive for S. aureus.

A successful fermentation in dry (unheated) sausages is necessary not only to produce a sausage with a desirable flavor and aroma, but also to control the growth of S. aureus. The practice of adding part of a previously fermented batch of sausage to the next batch of sausage mix as a source of LAB, instead of using a pure starter culture, was used by a number of companies during the period of this study. We used this approach before we developed customized pure lactic starters. Eighteen to nineteen days after, stuffing samples were taken from large diameter salamis and analyzed for total type of LAB as well as S. aureus. Salamis with the highest LAB counts were chopped, vacuum-packed and kept refrigerated as starters for the next 20 days. This practice assured initial LAB counts of over 10^5/g salami mix and a decrease of the salami pH to <5.3 within 2-3 days. The AMI guidelines for 23.8°C (75°C) and 26.6°C (80°F) fermentation temperatures recommend that the pH should reach <5.3 within 60 hours, respectively.

B. Chemical Changes. Depletion of nitrite starts as soon as it is added to meat. The rate of depletion depends on product formulation, pH of the product, and time and temperature of the processing and post-processing condition (5, 14). The depletion rate doubled for every 12°C increase in temperature or 0.86 pH unit decease (14) and was directly proportional to the meat concentration (16). Product formulation may explain some of the differences in nitrite and in nitrosomyoblobin between salamis of Company A and B. The final product of Company A was composed of 23.6% beef, while Company B's product contained 16.5% beef. This may have resulted in more myoglobin in Company A's salami compared to Company B's since beef contains 0.3 to 1.0% myoglobin versus

pork with 0.06 to 0.4% myoglobin (15). The significant difference between Company A and B in moisture of their salamis during the ripening period probably reflects the differences in temperature and RH of their ripening rooms. Also, the lower pH of the salamis of Company A during the ripening period may have caused Company A's salamis to release moisture at a faster rate than those of Company B. Kramlich (10) stated that the pH of sausages after fermentation should be near 5.1 to ensure satisfactory removal of moisture from the sausages on drying. The difference in percent moisture among the three diameters of salami is considered due to the casing diameter. The latter has a significant effect on the rate of drying for most products (1, 17). With larger diameters, drying occurs more slowly. The diameter effect on the rate of moisture loss would also explain the difference in a_w among the salami diameters. Although there was a significant moisture loss in the salamis during the fermentation period, a significant decease in a_w did not occur until the ripening period. This would indicate that large amounts of moisture must be lost to yield small changes in a_w. Niskanen and Nurmi (13) observed no significant change in a_w during the first 3 days of their dry sausage production. The difference in mean values for color among the diameters were due to the difference in moisture content among the diameters; there were no color differences once color data were adjusted for moisture. Keller et al (9) found that increases in chemical components (protein, fat, ash, salt, lactic acid) during 45 days of drying of summer sausage were dependent on the rate of moisture removal from the sausages. Color like salt and lactic acid, is concentrated by removal of moisture.

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Table I. Comparison of Company A and Company B adjusted mean values for 10 variables recorded at eight time periods during salami processing*

Day	pH		Titratable Acidity (%)		Nitrite (ppm)		Color (ppm)		Brine (%)		a_w		Moisture (%)		Cocci (log_{10})		S. aureus (log_{10})		Lactics (log_{10})	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
0	6.07	6.09	0.30	0.29	140.0	140.0	13.7	11.9	6.1	5.7	0.955	0.949	[52.8	54.8]**	[3.78	4.26]	2.16	2.38	5.09	5.30
1	6.00	6.06	0.34	0.31	76.9	73.8	[44.0	36.9]	6.8	6.8	[0.955	0.947]	52.3	53.1	[4.95	5.89]	2.30	2.42	6.52	6.22
2	5.67	5.75	0.52	0.56	[39.7	61.6]	[58.9	50.6]	7.2	7.4	[0.951	0.942]	50.8	51.4	[5.67	6.57]	2.62	2.80	8.41	8.29
3	5.16	5.22	0.80	0.73	[14.5	25.5]	[69.7	59.3]	8.0	7.8	[0.948	0.939]	49.0	50.1	[5.15	6.16]	2.77	2.76	8.31	8.51
7	[4.78	4.92]	[1.04	0.94]	11.5	14.3	[65.3	54.8]	9.1	8.8	0.922	0.918	[44.6	46.7]	[4.55	5.76]	2.51	2.86	8.18	8.11
14	[4.58	4.76	1.35	1.09	11.3	14.2	67.5	60.7	11.3	10.5	0.898	0.898	[40.2	42.1]	[4.23	5.60]	2.61	2.90	8.17	8.03
21	4.38	4.52	1.39	1.11	9.0	10.8	80.3	72.2	[13.0	10.9]	[0.905	0.905	[38.1	41.4]	[4.24	5.54]	2.11	2.71	8.19	8.08
28	4.43	4.50	1.51	1.30	10.2	8.7	78.8	77.6	11.2	10.5	0.902	0.907	[37.3	42.4]	[4.31	4.95]	2.04	2.92	8.19	7.76

* Data were adjusted for time (year) of salami manufacture by the methods of analysis of covariance.

** Mean values enclosed in brackets are significantly different at P<0.01.

Table 2. Three highest counts of S. aureus ($\log_{10}$/g) by day, salami diameter and company on each test day.

		0	1	2	3	7	14	21	28
Company A		4.45[A]	4.04[C]	4.18[A]	4.65[A]	5.28[A]	4.23[G]	4.54[A]	NT
Diameters	Small	3.08[B]	3.60[B]	3.79[D]	4.41[E]	3.54[E]	4.00[E]	3.18	
	N = 34	2.70	3.36	3.78[E]	3.59[F]	3.48[F]	3.60[C]	3.08	
		4.71[F]	3.48[B]	4.89[D]	4.43[H]	4.28[B]	3.98[H]	3.85[B]	NT
	Medium	4.08[B]	3.48[C]	4.36	3.99[G]	3.92[D]	3.94[C]	3.56	
	m = 31	3.90	2.94[G]	4.08[E]	3.58	3.68[G]	3.85[E]	2.70	
		3.38	4.00	4.57[B]	4.95[G]	4.52[G]	5.18[G]	3.65[B]	3.68[B]
	Large	3.32[B]	3.11	4.23[G]	4.15	4.43[B]	4.26[B]	3.40[I]	2.00[I]
	N = 28	3.04	3.08[B]	4.04[F]	4.11[E]	3.68[F]	3.85[C]	2.48[C]	2.00[C]
Company B		4.75[M]	4.66[M]	5.48[P]	5.00[P]	6.04[P]	5.98[P]	4.46[T]	NT
Diameters	Small	4.08[N]	4.45[P]	5.00[R]	4.83[S]	4.91[R]	4.95[R]	3.78[M]	
	N = 39	3.46[P]	3.69[O]	4.61[S]	4.34[R]	4.52[S]	4.34	3.78[U]	
		4.40	4.51[M]	5.11[R]	5.00[R]	4.45[O]	4.34[T]	3.83	NT
	Medium	4.34[M]	3.58[V]	4.04[S]	3.83[S]	4.26[T]	4.30[U]	3.48	
	M = 41	4.00[N]	3.53[N]	3.49[O]	3.43[W]	4.11[S]	4.28[R]	3.45[T]	
		4.85[M]	4.78[X]	5.08[S]	4.32[T]	4.23[S]	4.96[S]	4.31	4.48[N]
	Large	4.34[X]	4.36[M]	4.28[R]	4.18[P]	4.15[W]	4.41	4.26[Y]	3.96[M]
	N = 45	3.30[U]	4.15[V]	4.00[Y]	4.18[S]	3.96[P]	4.50[T]	3.95[M]	3.78[Y]

N = samples size; NT = Not tested; ABC = S. aureus counts which share the same letter superscript indicate salamis produced on the same day.

Table 3. Counts of S. aureus and lactics ($\log_{10}$/g), pH values, and titratable acidity percentages of the five high count lots (HCL[1]) of salami

			S. aureus		Lactics		pH		TA	
Co.	Dia-meter	Test-day	HCL	Mean[2]	HCL	Mean	HCL	Mean	HCL	Mean
A	Small	0	4.45[c]	2.23	5.83	5.05	6.1	6.08	NT[3]	0.29
		1	NT	2.23	NT	6.42	NT	6.06	NT	0.32
		2	4.18[b]	2.55	6.48[a]	8.12	5.8	5.71	NT	0.52
		3	4.65[b]	2.61	8.48	8.06	5.6	5.25	NT	0.77
		7	5.28[c]	2.54	7.28	7.99	5.6	4.92	NT	1.01
		14	NT	2.40	6.88	7.99	5.3	4.72	NT	1.21
A	Large	0	2.48	2.37	3.40[c]	4.95	6.2	6.04	0.18[a]	0.33
		1	2.30	2.30	6.18	6.27	6.1	6.02	0.22	0.37
		2	3.11	2.66	7.87	8.35	6.0	5.71	0.26	0.52
		3	4.95[a]	2.91	8.18	8.38	5.5	5.16	0.35	0.85
		7	4.52[a]	2.79	7.44	8.23	4.6	4.69	0.86	1.15
		14	5.18[c]	2.54	7.59	8.21	4.3	4.44	1.68	1.51
B	Small	0	3.46	2.48	4.23[a]	5.35	6.1	6.10	0.15[a]	0.29
		1	4.45[b]	2.46	6.71	6.50	6.1	6.05	0.20	0.33
		2	5.48[c]	2.84	7.83	8.36	5.9	5.66	0.28	0.56
		3	5.00[b]	2.56	8.20	8.57	5.5	5.22	0.35[a]	0.70
		7	6.04[c]	2.83	8.04	8.24	5.1	4.97	0.58[a]	0.89
		14	5.98[c]	2.81	8.23	8.06	4.8	4.79	0.97	1.01
B	Medium	0	2.60	2.52	4.79	5.20	6.2	6.12	0.24	0.28
		1	2.00	2.32	5.93	6.10	6.2	6.11	0.29	0.30
		2	5.11[c]	2.59	8.20	8.27	5.9	5.67	0.40	0.55
		3	5.00[c]	2.54	8.61	8.30	5.2	5.16	0.65	0.71
		7	3.00	2.66	7.87	7.91	4.8	4.89	0.97	0.93
		14	4.28[b]	2.57	7.71	7.82	4.6	4.70	1.26	1.07
B	Large	0	2.48	2.37	5.54	5.12	6.2	6.10	0.29	0.30
		1	2.30	2.33	5.97	5.99	6.2	6.12	0.40	0.32
		2	5.08[c]	2.44	8.15	8.41	6.0	5.66	0.47	0.59
		3	4.18[a]	2.45	8.89	8.51	5.4	5.20	0.76	0.78
		7	4.23[b]	2.49	8.20	8.10	5.0	4.85	1.09	1.03
		14	4.96[b]	2.62	8.38	8.03	5.0	4.65	1.26	1.26

[1] Lots which had S. aureus counts $>10^5$/g on one or more test-days

[2] Company mean response value for salamis of given diameter on given test-day

[3] Not tested

[abc] Indicates values which are, respectively, 2-2.4, 2.5-2.9 and ≥ 3 standard deviations from their mean