Effect of nitrate, nitrite and a starter culture on the growth of Yersinia enterocolitica in dry sausage

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Introduction

Animals, especially pigs, have shown to be reservoars of Yersinia enterocolitica, which causes gastrointestinal and other types of infections in man (1, 5, 10). Several surveys about the occurrence of the organism in caecal contents or faces of animals at slaughterhouse have been made. 3.7 % of the samples taken from pigs were positive in Canada (10), 4.0 % in Japan (2), and 0.5- 39.3 % in Europe (6, 8, 9, 11, 12). Furthermore in average 11.7 % of pigs were shown to carry the organism in their throaths in two West-European studies (6, 11). In a Japanese study (3) 7.9 % of faces samples of cows at slaughter were positive to Y. enterocolitica as were 11.3 % in a study made in the Federal Republic of Germany (12). Leistner et al. (12) isolated the organism from 34.5 % of pork and 10.8 % of beef meat samples. In a Japanise study (3) 24.6 % of beef meats sampled were positive.

The purpose of this study was to evaluate the possible health hazard associated with the use of raw material contaminated with Y. enterocolitica for the manufacturing of dry sausage.

Material and Methods

The dry sausage was prepared using normal commercial manufacturing practice according to the following recipe: beef 47 %, pork 24 %, pork fat 25 %, NaCl 3 %, glucose 0.6 %, seasonings 0.4 %. The combinations of additives and bacterial cultures used for each batch are shown in table I. These additions were mixed with 2 kg of basic sausage mix in a kitchen blender. Three sausages were made from each batch.

The following Y. enterocolitica strains were used in the study: ATCC 27729 obtained from the American Type Culture Collection, Rockville, Maryland; and UCLA 151 obtained from the California State Department of Health, Berkeley, California. The both strains were human clinical isolates, the former serotype O:8, the latter O:9. Overnight culture (at 30°C) in nutrient broth was diluted in 3 ml of saline and used for 2 kg of the sausage mix.

Duploferment 66, manufactured by Rudolf Müller and Co, Giessen, Federal Republic of Germany, was used as a starter culture. It contains micrococci and lactobacilli and was used according to the manufacturer´s recommendations (10 ml of the final dilution per 2 kg of sausage mix).

Samples were taken for the following quantitative bacterial analyses from the raw sausage mix and on the third, seventh and fifteenth day of ripening and drying: Determination of Y. enterocolitica, SS- and MacConcey´s agars (30°C/48 h) followed by cultivations on Urea- and Triple Sugar Iron-agars (30°C/24 h). Determination of micrococci, Staphylococcus medium 110 (37°C/48 h). Determination of lactobacilli, Rogosa-SL-agar (30°C/72 h anaerobically). All media were purchased from Difco. pH was analysed using Beckman Zeromatic pH meter equipped with Beckman combination electrode 39183 (Beckman Inc., Fullerton, California).Water activity (a_w) was measured with Vaisala Humicap HM 14 U hygrometer (Vaisala Oy, Vantaa, Finland).

Results

The results of the bacteriological analysis of the sausage samples are shown in table II.The raw sausage mix had pH-value 5.45 and a_w 0.97. After 15 days' ripening and drying the corresponding values were 5.20 and 0.90 for the sausages without the starter and 4.90 and 0.89 for those having the culture.

Discussion

A relatively high artificial contamination level of Y. enterocolitica was used in this study: Logarithmic counts in the raw sausage mix ranged from 4.95 to 6.15. The trend of rapid decline in the counts during the ripening and drying of the sausages was seen in each sample, also in those to which no starters or additives were used (sausages 1 and 6 in table 2). The decrease was, however, more rapid if nitrate or nitrite was used, and most

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Sausage	Y. enterocolitica strain	Starter culture used	$NaNO_2$ added (mg/kg)	KNO_3 added (mg/kg)
1	UCLA 151	-	0	0
2	"	-	150	0
3	"	-	0	300
4	"	+	150	0
5	"	+	0	300
6	ATCC 27729	-	0	0
7	"	-	150	0
8	"	-	0	300
9	"	+	150	0
10	"	+	0	300

table I

The additions that were made to the basic sausage mix. The food additives were added in 10 % water solutions.

	0 days			3 days			7 days			15 days		
Sausage	Y.e.	Mic.	Lac.	Y.e.	Mic.	Lac.	Y.e.	Mic.	Lac.	Y.e.	Mic.	Lac.
1	5.62	4.18	4.11	4.85	4.95	8.00	4.74	5.11	7.78	2.78	3.90	7.97
2	5.23	3.90	3.85	4.30	4.48	7.28	3.00	3.78	7.76	2.60	3.70	7.96
3	5.61	4.62	3.70	4.43	4.70	4.90	3.91	4.60	7.82	2.30	4.30	7.87
4	5.60	7.00	6.90	4.00	6.70	7.18	3.00	6.60	7.15	<2.00	6.49	7.28
5	5.18	7.11	6.83	4.48	6.70	7.28	3.00	6.20	6.91	<2.00	6.57	6.96
6	5.51	4.65	4.36	5.28	5.70	8.08	4.48	5.75	8.43	2.30	5.70	7.98
7	4.95	4.38	3.91	4.48	4.00	7.90	4.86	5.26	8.30	<2.00	3.60	7.34
8	5.46	3.61	4.04	5.84	5.30	8.00	4.00	3.48	7.78	3.04	5.04	8.08
9	6.04	7.36	6.81	4.90	7.48	7.99	3.00	7.04	7.54	<2.00	6.74	7.34
10	6.15	7.26	6.86	4.08	7.43	7.34	3.00	6.94	7.59	<2.00	6.62	7.04

table II

The logarithmic results of the quantitative bacterial analyses of the dry sausages during ripening and drying.

(Y.e.= Yersinia enterocolitica, Mic. = micrococci, Lac. = lactobacilli).

rapid if the starter culture combined with one of the additives was used. After 15 days Y. enterocolitica could not be detected from any of the samples of this type (numbers 4, 5, 9 and 10) using the surface plating method. The same result was obtained for the sample 7 to which 150 mg/kg $NaNO_2$ had been added. The decrease in the Y. enterocolitica population was quicker than what was found in case of Salmonella senftenberg in a previous study (7). Enrichment methods were not used in our study to detect Y. enterocolitica concentrations less than 100/g. The data in table 2 represents results from just one analysis of the single sample taken. More data should be available in order to be able to draw definite conclusions about the behavior and possible health hazard of Y. enterocolitica in dry sausage. According to this study there still can be detectable amounts of the organism in the artificially contaminated dry sausage after 15 days' ripening and drying period. This is the case especially if the starter culture with nitrate or nitrite is not used.

The behavior of the sausage as far as pH, a_w and micrococci and lactobacilli counts are concerned followed the pattern found in former studies (4, 7).

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