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ISOLATION AND CHARACTERIZATION OF MICROCOCCACEAE FROM BULGARIAN RAW-DRIED SAUSAGE

R.N.BORPUZARI and K.BOSCHKOVA

Higher Institute of Food and Flavour Industries, Department of Technology of Meat and Fishes, Plovdiv, Bulgaria

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INTRODUCTION

'Lukanka' is a typical Bulgarian raw-dried sausage, traditionally manufactured by a "natural flora" fermentation, which is highly valued for its characteristic flavour and aroma. The microorganisms inherent to the meat and the spices used in the production of Lukanka play a decisive role in the production of the characteristic flavour and aroma of this product. *Micrococcaceae* and lactic acid bacteria are the two important groups of microorganisms responsible for the fermentation of raw-dried sausages (Palumbo and Smith, 1977; Liepe, 1982). Some work have been done on the distribution of lactic acid bacteria in Lukanka, but literature on the dynamics of *micrococcaceae* during different stages of its production is scanty. Therefore, this study was undertaken to investigate the distribution of microorganisms in Lukanka, with emphasis on the *micrococcaceae*, with the aim of isolating and selecting a few strains of *micrococci* for possible use as starter culture in the industrial production of Lukanka.

MATERIALS AND METHODS

Processing conditions

The bacterial populations of five batches and yeasts populations of three batches, of Lukanka manufactured by natural fermentation were studied. The composition of the sausage mixture was (%, w/w)- beef (60), pork lean (20), pork breast (20), NaCl (2.4), NO_3 (0.016), sucrose (0.2), black pepper (0.3), and cummin (0.3). After the initial fermentation phase (10-12°C, relative humidity, RH 85-90%, air circulation rate 0.2-0.3m/sec, 24 hours), the sausages were ripened for 10 to 12 days at 10-15°C with the RH at 80-85% and an air circulation rate of 0.05-0.1m/sec. They were then ripened for a further 10 to 12 days at the same temperature and air circulation rate, but with the RH at 75-80%. Finally, the sausages were ripened at the same temperature but with the RH at 70-75% and an air circulation rate of 0.1m/sec until the products were ready for marketing. On the sixth and 13th day of ripening the sausages were pressed for 12 to 24 hours to force moisture from the core of the sausage mass to the surface and so facilitate drying. That processing gives the characteristic shape to Lukanka.

Microbial analysis

Samples for microbial analysis were taken from the sausage mix (A), after the initial fermentation phase (B), and on the third (C), sixth (D), 13th (E) and 28th (final) day of ripening (F). Ten gram sample from different parts of the sausage was weighed aseptically and blended with 90ml of 0.1% peptone water. Serial dilutions were prepared from the homogenate.

Total viable counts (TVC) were determined on plate count agar (PCA) pH7.0. The counts of *micrococcaceae* were made on mannitol-salt agar (MSA) of pH7.2 (Chapman, 1945). The lactic acid bacteria were counted on double layer

MRS agar pH6.4 (de Mann *et al.*, 1960), and the counts of yeasts were made on Czapek-Dox agar (Harrigan and McCance, 1976). All plates were incubated at 30°C for three days. The numbers of colonies were expressed as $\log_{10}$cfu/g of sample.

For isolation of *micrococcaceae*, about 20% of the colonies growing on MSA were randomly selected (Ordonez, 1979). A total of 354 strains thus isolated were maintained on basal medium (Selgas *et al.*,1988) with periodic subculturing at two months interval. For characterization and differentiation of the isolated strains into *micrococci* and *staphylococci*, they were grown in mannitol-salt broth for 18 to 24 hours and the active cultures were subjected to the following tests: cell morphology, Gram's staining, catalase reaction (Harrigan and McCance, 1976), oxidase reaction (Faller and Schleifer, 1981), differentiation of oxidative and fermentative metabolism of glucose in modified Hugh and Liefson's medium (Recommendations, 1965), growth on furazolidone (FTO) agar (von Rheinbaben and Hadlok, 1981) and lysostaphin susceptibility test (Langlois *et al.*, 1988).

RESULTS AND DISCUSSION

Changes in microbial population during different stages of Lukanka production are presented in Table 1. The TVC and the numbers of *lactobacilli* and *micrococcaceae* slowly but steadily increase, from the initial mean value of $\log_{10}$4.86, 4.07 and 4.12cfu/g respectively, until the third day of ripening. They then increase to rapidly reach their maximum numbers, of $\log_{10}$5.45, 5.20, and 4.67cfu/g respectively, on the sixth day of ripening. The TVC and the *lactobacilli* counts decreased thereafter until the 13th day of ripening, and then increased slowly. The number of *micrococcaceae* decreased from the sixth day of ripening to $\log_{10}$4.33cfu/g in the finished product. The number of yeasts was significantly lower than the bacterial counts throughout the production process. Yeasts grew from the low initial mean value of $\log_{10}$2.91cfu/g in the sausage mix to reach the maximum mean value of log 4.26cfu/g on the 13th day of ripening. Thereafter yeast numbers slowly declined.

The initial numbers of *micrococcaceae* was higher than those of the *lactobacilli*. However, the growth rate of the *micrococcaceae* was significantly lower than that of the lactobacilli, so after the initial fermentation phase the lactobacilli outnumbered the *micrococcaceae*. Reuter *et al.*(1968) also observed similar changes in the growth rates of micrococci and lactobacilli in quick-ripened Germany dry sausages.

The maximum numbers of *micrococcaceae* were found on the sixth day of ripening. Lactic acid bacteria were the predominant organisms throughout ripening. Similar findings have been reported by Nurmi (1966) and Genigeorgis (1976). In general, the development of the flora of Lukanka was similar to that observed in Spanish dry fermented sausage by Selgas *et al.* (1988), but the numbers of the flora observed in our study were smaller. However, we observed *micrococcaceae* at $\log_{10}$4.33cfu/g in the finished product whereas Selgas *et al.* (1988) did not find any *micrococcaceae* in their products after 25 days of ripening.

Of the 354 strains of *micrococcaceae* isolated from the MSA plates, 213 (60.17%) were classified as *micrococci* and the remaining 141 (39.83%) as *staphylococci*. The percent distribution of *micrococci* and *staphylococci* during different stages of the sausage production is shown in Figure 1. It is seen from the figure that the percent incidence of *micrococci* decreased, from the initial value of 17.37% until the sixth day of ripening, and then with a sudden increase reached the maximum incidence of 28.17% on the 13th day of ripening. In the finished product the fraction of *micrococci* was 18.78%.

For *staphylococci*, the maximum fraction was observed in the sausage mix (31.20%). There was then a rapid fall in the fraction of *micrococci* until the third day of ripening. Subsequently, the fraction increased to reach 24.82% on the 13th day of ripening and then declined slowly. The fraction of *staphylococci* in the finished product was 21.98%.

CONCLUSION

The TVC and the counts of *micrococcaceae* and lactic acid bacteria in the raw, dried sausage, Lukanka, increased from the sausage mix to the sixth day of ripening. After that stage, the TVC and the *micrococcaceae* counts decreased. In contrast, the *lactobacilli* counts decreased until the 13th day of ripening, and then increased. The number of yeasts increased from the sausage mix to the 13th day of ripening, and then decreased.

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Table 1. Dynamics of microbial population during different stages of "Lukanka" production (log_{10} cfu/g).

	Stages of isolation					
	A	B	C	D	E	F
TVC						
Min	4.28	4.00	4.15	5.07	5.08	5.00
Max	5.30	5.20	5.34	5.76	5.68	5.74
Mean ± SE	4.86 ±0.06	4.96 ±0.06	5.10 ±0.05	5.45 ±0.05	5.35 ±0.04	5.36 ±0.05
Micrococcaceae						
Min	3.00	3.08	3.48	4.00	3.78	3.60
Max	4.57	4.60	4.78	5.08	4.86	4.70
Mean ± SE	4.12 ±0.08	4.28 ±0.06	4.43 ±0.07	4.67 ±0.07	4.48 ±0.05	4.33 ±0.05
Lactic acid bacteria						
Min	3.00	3.58	3.95	4.95	4.96	4.95
Max	4.60	4.90	4.90	5.50	5.39	5.41
Mean ± SE	4.07 ±0.08	4.51 ±0.07	4.65 ±0.06	5.20 ±0.04	5.14 ±0.03	5.15 ±0.04
Yeast						
Min	1.00	2.83	3.28	3.84	3.90	3.70
Max	3.11	3.60	3.60	4.11	4.50	4.24
Mean ± SE	2.91 ±0.06	3.25 ±0.08	3.36 ±0.06	3.95 ±0.03	4.26 ±0.06	4.07 ±0.01