MICROBIAL CHARACTERISTICS OF MUTTON DRY FERMENTED SAUSAGES AS AFFECTED BY THE USE OF COMBINATIONS OF STARTER CULTURES

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Abstract—The microbiological of mutton fermented sausages with combinations of starter cultures were evaluated to determine their quality characteristics during ripening and storage. These sausages were manufactured with mutton and tail fat obtained from sheep fed in Inner Mongolia, PRC. Two groups of fermented sausages manufacture using the same technology were named control group (CO) and starter culture group (SC). In this study, Lactic acid bacteria counts in sausages made with combinations of starter cultures increased to 8 logcfu/g and remained at this level throughout the storage period. The total bacteria counts in the SC were lower ($P < 0.05$) than in the control group after drying and storage time. The *Micrococci-staphylococci* counts in the samples inoculated with combination of starter culture were significantly lower ($P < 0.05$) than in the control during ripening.

***Index Terms*—microbiological characteristics, starter cultures combination and mutton fermented dry sausages**

I. INTRODUCTION

Fermented sausages are the result of biochemical, microbiological, physical and sensorial changes occurring in a meat mixture during ripening under defined conditions of temperature and relative humidity (RH)（Casaburi et al., 2007). Nowadays, due to changes in shopping and consuming habits, the problem of safe preservation in the meat industry has become more complex and today's products require a longer shelf life and greater assurance of protection from microbial spoilage. The commercial starter cultures in China are not always able to compete well with natural fermentation，therefore, their use often results in losses of desirable sensory characteristics (Leroy, Verluyten, and De Vuyst, 2006). For this reason such artificial fermented sausages are often of superior quality compared to those inoculated with industrial starters and possess distinctive qualities due to the technology used and to the properties of the raw meat (Moretti et al., 2004). Therefore, appropriate starter cultures have to be selected according to the specific formulation of the batter and technology of fermentation since environmental factors will interact to select a limited number of strains that are competitive enough to dominate the process. In order to make the ideal starter culture for any particular technology and recipe, it is necessary to understand the properties required, and to have tools to improve the properties of the culture.

The main microbial groups of technological interest isolated in spontaneously fermented sausages are lactic acid bacteria (LAB) (Corbiere Morot-Bizot, Leroy, & Talon, 2006). These LAB have a positive effect on the hygienic properties of the product, inhibiting pathogenic and spoilage flora by acidification and by the production of antimicrobials (Villani, Pepe, Mauriello, Salzano, Moschetti & Coppala, 1994). Such as, *Lactobacillus Pentosus* was used as the acidifying component of the starter culture combinations in order to assure appropriate acidification of the fermented sausages during the ripening and storage periods.

Staphylococcus contribute to the development of colour by reducing nitrate to nitrite and participate in the development of flavours of dry fermented sausages (Demeyer et al., 1986). They influence the composition of non-volatile and volatile compounds mainly by degrading free amino acids and inhibiting the oxidation of unsaturated free fatty acids (Sondergaard and Stahnke, 2002). Moreover, recent publications regarding the microbiological characteristics of fermented sausages have primarily focused on pork products (Severini, De Pilli, & Baiano, 2003; Valencia, Ansorena, & Astiasarán, 2006a). Only a limited number of papers have dealt with fermented mutton sausages (Zapata, Matos, Teles, Guedes & Vasconcelos, 1992; Cattaneo, Stella, Ripamonti, Marossi, & Donizetti, 2003).

The aims of this work were to use lean mutton and sheep tail fat as raw material for manufacturing fermented sausages in order to evaluate the effect of the starter culture on the microbiological properties of mutton fermented dry sausages.

II. MATERIALS AND METHODS

2.1 **Starter culture formulation**

a strain of *Staphylococcus carnosus* isolated from traditionally cured meat in Hunan province (Southern China) was used in combination with an acidifying strain of *Lactobacillus pentosus and Pediococcus pentosaceus* as a starter culture for the production of fermented sausages, provided by Laboratory of Meat Science, College of Food Science and Nutritional Engineering, China Agricultural University.

2.2 **Mutton fermented dry sausage manufacture**

All the sausages were manufactured on the same day and using the same technology at the Laboratory of Meat Science, College of Food Science and Nutritional Engineering, China Agricultural University. The sausage formulation included 78% lean mutton, 22% tail fat, 2.5% salt, 0.5% glucose, 0.5% sucrose, 100ppm sodium nitrate, and 70ppm sodium nitrite. After chopping and mixing the ingredients, the mixture was divided into two batches, which were named control (CO), starter culture (SC). the sausage mixtures were manually stuffed into natural sheep casings (26-28mm) and placed in a constant temperature and humidity incubator. The relative humidity (RH) and temperature are reported in Table 1. At each sampling period［ripening days 0, 1.5, 3, 5, 7 and storage weeks 1 (days 14)，2 (days 21), 4 (days 35)］two sausages from two batch were taken for microbiological analysis.

2.3 **Microbial analyses**

Ten grams of each sample were taken aseptically from the stored packages and were 10-fold diluted in sterile 0.1% peptone water and 0.85% NaCl according to GB/T4789.35-2003 (China). The solution was homogenised for 2min in a stomacher. Serial decimal dilutions were made in sterile peptone water and duplicate 1ml or 0.1ml samples of appropriate dilutions were poured or spread onto total count and selective agar plates.

The microbiological analyses made on the samples were:

Aerobic mesophilic bacteria determined on Plate Count Agar (PCA) incubated at 30°C for 72h, LAB were enumerated on MRS Agar in anaerobic conditions after 72h at 30°C and Micrococcaceae on Mannitol Salt Agar (MSA) after 72h at 30°C.

2.4 **Statistical analysis**

Data were statistically analysed by means of One-way analysis of variance (ANOVA). When main effects were significant, the means were separated by Fisher's least significant difference test at 5% (LSD 0.05) (SPSS 12.0). The level of significance ($P \leq 0.05$) was used for all comparisons. Mean values and standard error of the means (SEM) were reported.

III. RESULTS AND DISCUSSION

The results of the microbiological analyses are reported in Table 2. Counts of lactic acid bacteria (*LAB*) were initially significantly lower in the control ($p<0.05$) than in the SC after clustering (0d) due to the inoculation of both *Lb.pentosus* and *P. pentosaceus* starter strain (Bover-Cid et al., 2001). In two groups lactic acid bacteria (*LAB*) dominated the microflora from the beginning of the fermentation and after 1.5 days were above 8 logcfu/g and maintained this level during ripening. Due to the good adaptation of *LAB* to the meat environment and their faster growth rates during fermentation and sausage ripening, they become the dominant microbe (Drosinos, Mataragas, Xiraphi, Moschonas, Gaitas, & Metaxopoulos, 2005). During sausage storage time, *LAB* counts of the sausage inoculated with combined starter cultures were significantly higher than of the control ($p<0.05$). *LAB* counts of two groups decreased significantly ($p<0.05$) and maintained 8 logcfu/g level at 35 days. In general, the stability found for the counts of *LAB* during ripening and storage time could be explained by *LAB* becoming the dominant microbe and packaging in anoxic environments retards microbial growth and delayed spoilage due to slow proliferation of bacteria capable of tolerating anaerobic conditions (Martínez, Djenane, Cilla, Beltrán, & Roncalés, 2006).

Initial *total bacterial* counts were 6 logcfu/g in two groups (Table 2). Prior to d1.5 of fermentation there was a rapid increase in the control and other group (about 8 logcfu/g). During ripening (from d5 to d7), total bacterial counts were 7logcfu/g in group inoculated with starter SC, which was significantly lower ($p<0.05$) than the control group. *Total bacterial* counts of the control during storage were higher ($p<0.05$) than the sausage inoculated with combination of starter cultures. The growth of *Total bacterial* counts in the SC appeared to be significantly affected by acidification. Probably due to the strong competitive ability of lactic acid bacteria on the rest of the endogenous bacteria, which assured safe preservation of fermented sausages. Lactic acid bacteria

suppress the growth of other bacteria by producing organic acids and various antibacterial metabolic products (Holzapfel, Guisen, & Schillinger, 1995).

Initial counts of *Micrococci-staphylococci* in sausage inoculated with the starter culture combinations were 5 logcfu/g and higher (p<0.05) than in the control after clustering (0d) (Table 2), due to inoculating with *Staphylococcus carnosus*. From fermentation to ripening, counts of *Micrococci-staphylococci* in SC increased rapidly to about 6 logcfu/g and were lower (p<0.05) than in control (7 logcfu/g). The growth of *Micrococci-staphylococci* in the samples inoculated with starter culture appeared to be significantly affected by acidification or by the inability of the starter to compete with the autochthonous microbe. This was in agreement with Lizaso et al. (1999) who considered acidification to be the main cause of *Micrococci-staphylococci* inhibition in dry fermented sausages.

IV. CONCLUSION

The use of starter culture combinations resulted in a change in some of the microbial properties. Lactic acid bacteria population in inoculated sausages increased to 8 logcfu/g and remained high during the storage period effectively suppressing the growth of pathogenic and spoilage microbe by acidification. Therefore the use of combinations of starter cultures could improve the safety, the quality attributes and shelf life of mutton fermented sausages.

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Table 1: Technological parameters for preparation of dry fermented mutton sausages

Stage	Temperature (°C)	Time	RH (%)
Curing	0-4	12-15 h	vacuum
Fermenting	24-25	0-1.5 days	95-96
Drying I	14-15	1.5-3 days	85-90
Drying II	14-15	3-5 days	75-80
Ripening	14-15	5-7 days	65-70
Storage	18-20	8-35 days	vacuum

Table 2 Microbiological characteristics during the ripening and storage of dry fermented mutton sausages[1,2,3]

Ripening and storage time（days）		Lactic acid bacteria (logcfu/g)		Total bacterial (logcfu/g)		Micrococci-staphylococci (logcfu/g)	
		CO	SC	CO	SC	CO	SC
Ripening time	0d	7.03 ± 0.02^{aA}	8.13 ± 0.03^{bA}	6.09 ± 0.50^{aA}	6.98 ± 0.08^{bC}	2.99 ± 0.09^{aA}	5.92 ± 0.17^{bB}
	1.5d	8.80 ± 0.01^{aE}	8.96 ± 0.02^{bE}	8.52 ± 0.10^{aD}	8.61 ± 0.09^{aF}	6.45 ± 0.03^{bE}	5.93 ± 0.05^{aB}
	3d	8.81 ± 0.0586^{aE}	8.83 ± 0.01^{aCD}	8.36 ± 0.02^{bCD}	7.93 ± 0.02^{aDE}	7.00 ± 0.00^{bF}	6.41 ± 0.07^{aD}
	5d	8.63 ± 0.02^{aD}	8.84 ± 0.01^{bCD}	8.14 ± 0.00^{bC}	7.88 ± 0.02^{aD}	6.33 ± 0.01^{bE}	6.14 ± 0.04^{aC}
	7d	8.70 ± 0.10^{aD}	8.77 ± 0.07^{aC}	8.15 ± 0.13^{bC}	7.99 ± 0.05^{aE}	7.24 ± 0.08^{bG}	6.79 ± 0.01^{aE}
Storage time	14d	8.41 ± 0.01^{aC}	8.87 ± 0.05^{bD}	7.41 ± 0.03^{bB}	6.65 ± 0.05^{aB}	6.01 ± 0.04^{bD}	5.97 ± 0.01^{bB}
	21d	8.39 ± 0.01^{aC}	8.65 ± 0.03^{bB}	7.54 ± 0.02^{bB}	6.45 ± 0.02^{aA}	4.33 ± 0.31^{aB}	5.31 ± 0.07^{bA}
	35d	8.02 ± 0.09^{aB}	8.68 ± 0.08^{bB}	7.44 ± 0.01^{bB}	6.47 ± 0.02^{aA}	4.93 ± 0.03^{aC}	5.19 ± 0.01^{bA}

[1] Mean ± Standard Deviation.
[2] Means within the same row with different superscript upper case letters are different ($p<0.05$).
[3] Means within the same column with different superscript lower case letters are different ($p<0.05$).