Nitrate Reduction and Pigment Formation of Chinese-Style Sausage Mixes Caused by Micrococcaceae

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ABSTRACT : This study investigated the nitrate reduction ability of Micrococcaceae on pigment formation in Chinese-style sausage. One hundred ppm sodium nitrite and 150 ppm sodium nitrate was added asepticly to ground pork which was then inoculated with 10^7 CFU/g of either *Micrococcus varians*, *Staphylococcus carnosus* or *Staphylococcus xylosus*. All samples were cured at 20℃ or 30℃ for 3 days and then color, residue nitrite, nitrosyl pigment and residue nitrate were determined. The results indicated that samples inoculated with *S. xylosus* had higher a- and b- values due to nitrate reduction and pigment production after 3 days curing and these values were higher at the higher curing temperature. The nitrosyl pigment of the samples with *S. xylosus* had highest values after 3 days curing at both 20℃ and 30℃. However, sample inoculated with *S. carnosus* and *S. xylosus* had lower nitrate contents than the sample inoculated with *M. varians*. At 30℃ as well as *S. carnosus* and *M. varians* had a stronger decreasing in nitrate concentration during curing at 20℃. Moreover, samples inoculated with *S. xylosus* and *S. carnosus* had a higher residual nitrite content during curing at 20℃ or 30℃. In conclusion, two Staphylococci strains tested were most optimum starter cultures for improving pigment formation during Chinese-style sausage curing. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 8 : 1173-1177*)

Key Words : Micrococcaceae, Sausage, Nitrosyl Pigment, Nitrate Reduction

INTRODUCTION

Starter cultures have been used for many years to improve safety and quality, decrease fermentation time, and prolong shelf life of meat products (Geisen et al., 1992; Garriga et al., 1987; Bacus and Brown, 1981). However, most Chinese-style sausages are neither cured nor fermented in Taiwan. The procedure used to manufacture these products is quite simple. After ingredient mixing and stuffing, these products are dried at an elevated temperature (46 to 52℃ for 4 to 6 hrs). Therefore, due to high water activity (>0.90) and low acid production (pH>6.0), one month of shelf-life for these products can be expected if stored at 10℃. To improve the flavor and color of Chinese-style sausage, this research examined three starter culture strains for their ability to enhance these properties of sausages.

Although, redness of sausage was necessary for consumer appeal for these products there is little information available concerning the role of starter cultures in the area of color development of Chinese-style sausage. In previous investigations, this laboratory reported that the predominant bacteria in Chinese-style sausage are *Micrococcus* and to a smaller lesser *Staphylococcus* (Guo and Chen, 1991). These microorganisms belong to the family Micrococcaceae and they are also important in Western-style sausage production (Lucke, 1986; Jean, 1976). Published reports on the role of Micrococcaceae in color development and preservation can be summarized as follows: 1) They reduce nitrate to nitrite thus improving color and flavor of sausage (Geisen et al., 1992). 2) They contribute to the NADH/NADPH reduction system for reducing Met-myoglobin (Morita et al., 1994). 3) They produce catalase that decreases meat darkness and lipid oxidation arising from H_2O_2 (Juven et al., 1988; Rhee et al., 1987).

The purpose of this study was to investigate the nitrate reducing ability and pigment formation of Micrococcaceae on during Chinese-style sausage curing at 20℃ or 30℃.

MATERIALS AND METHODS

Starter inoculation

All microbiological strains were obtained from the Culture Collection and Research Center (CCRC, Taiwan). *M. varians* (CCRC 12272) was inoculated on MYP (Difco, USA) medium (30℃, 24 hrs), *S. carnosus* (CCRC 12922) and *S. xylosus* (CCRC 12930) were inoculated on Mannitol Salt Agar (37℃, 24 hrs). Colonies were washed with sterilized distilled water (SDW), resuspended in SDW and the absorbance adjusted Optical Density (O.D. $_{660\ nm}$=2.0) for beaker sausage inoculation (10^7 CFU/g meat).

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Preparation of beaker sausage

Frozen pork ham was thawed at 4℃ for 24 hr, cooked in water at 100℃ for 3 min and then surface of cooked ham was sterilized by flaming with 95% (v/v) alcohol. Before grinding, the burned pork ham surface was trimmed with a sterile knife. All sausages were made of 80% (w/w) ground lean pork ham (16 mm plate) and 20% (w/w) pork back fat (diced into 0.8 cm^3 cubes). Sausage raw materials were mixed with NaCl (1.5%, w/w), monosodium glutamate (0.5%, w/w), sucrose (2%, w/w) 100 ppm (w/w) $NaNO_2$ and 150 ppm (w/w) $NaNO_3$ then inoculated with either *M. varians*, *S. carnosus* or *S. xylosus* and used as samples of three different treatments. All ingredients of individual treatments were mixed and stuffed into sterilized beakers (70 mm×25 mm inside diameter), and designated as beaker sausage samples. The samples were incubated at 20℃ and 30℃ for 1, 2, and 3 days.

Measurement of color and nitrosyl pigment

The color of beaker sausages was expressed by the a-(redness), and b-(yellowness) values using the Handy Colorimeter (NR-300, Nippon Denshoku, Japan).

Nitrosyl pigment was extracted with acetone and distilled water and the optical density was measured on the extract at 540 nm (Ockerman, 1981) using a spectrophotometer (U-3210, Hitachi, Japan).

Nitrite and nitrate contents

A five grams of sample of the beaker sausage were homogenized, heated and its color was developed with a Griess solution. The nitrite content was measured by O.D. at 540 nm and calculated as described by Ockerman (1981). The nitrate content was determined by using a HPLC instrument (Model-6200, Hitachi, Japan) with Vydac 300 IC, 405 anion exchange column (50×4.6 mm i.d.), UV detector at 214 nm and 10 mM phosphate buffer saline (pH 6.0) of the mobile phase at 2 ml per min (Dennis et al., 1990).

Statistical analysis

Data were analyzed using a statistics software package (SAS, 1995). The ANOVA system was used to test the significance of treatment effect. When significant ($p<0.05$) differences were found, means were separated by the Duncans multiple range test.

RESULTS AND DISCUSSION

Change of color and nitrosyl pigment

The results of color tests for a-values of the samples are shown in figure 1. The samples inoculated with *S. carnosus* and *S. xylosus* had significantly higher a-values than the sample inoculated with *M. varians* ($p<0.05$) at 20℃ for 3 days. At 30℃, *S. xylosus* had the highest a- value among the treatments. These results also showed that the higher temperature also increased color development.

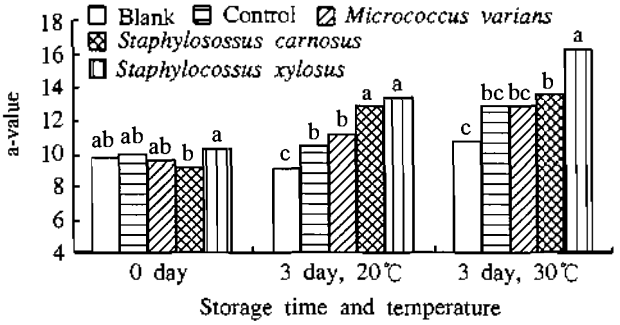


Figure 1. Change in a-value of beaker sausage after 3 days curing

The samples with *S. xylosus* had the highest b-values at 30℃ or 20℃ ($p<0.05$) because of the yellow pigment production from this strain. However, a fresh red color due to reduction ability was observed on the surface of the final products with *S. xylosus* when cured at 30℃ or 20℃ for 3 days (figure 2).

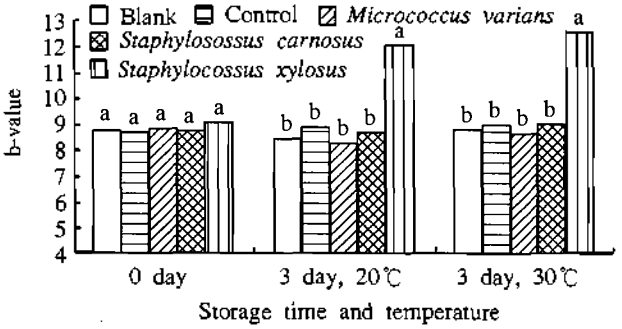


Figure 2. Change in b-value of beaker sausage after 3 days curing

The samples inoculated with *S. xylosus* had significantly ($p<0.05$) highest nitrosyl pigment content among the treatments (figure 3). Nearly twice (14.6 ppm for 20℃ and 16.3 ppm for 30℃) the nitrosyl pigment resulted from *S. xylosus* when the samples were evaluated after 3 days curing. *S. carnosus* also had slightly better pigment formation ability than that of *M. varians*. These results showed that usage of starter culture could increase the level of nitrosyl pigment in Chinese-style sausage due to the culture's reduction ability. The passive mechanism of reduction due to microbial inoculation might be similar to those reported by Arihara et al. (1993) and Fastman et al. (1990). They concluded that the oxygen consumption by bacteria and their affect on partial oxygen pressure

on meat surface allow the formation of metmyoglobin.

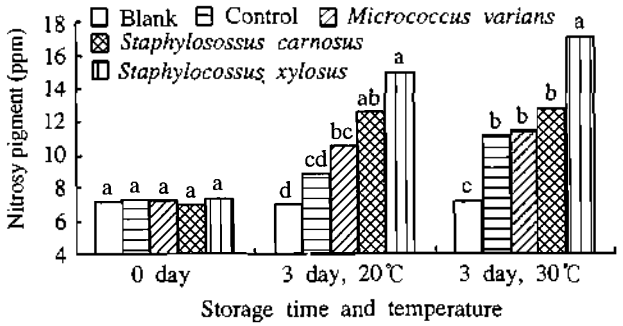
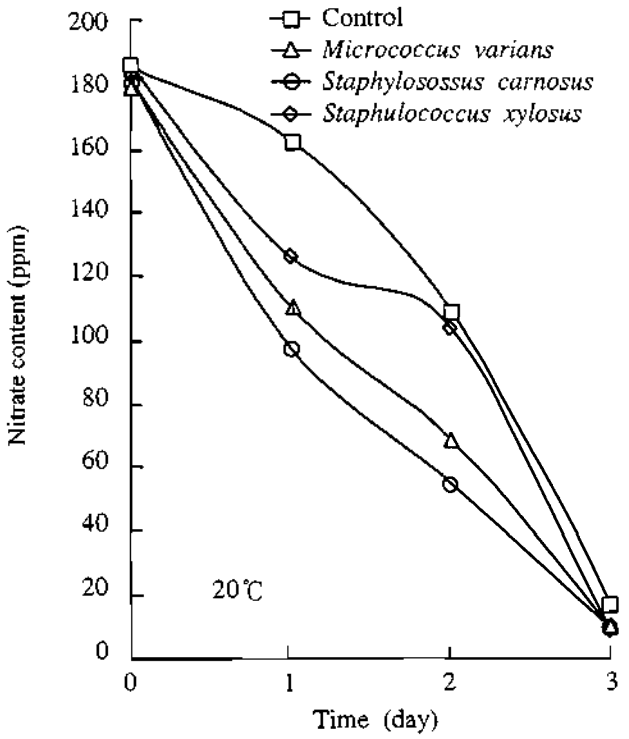


Figure 3. Nitrosyl pigment of beaker sausage after 3 days curing

Changes of nitrate and nitrite

Residual nitrate concentrations in samples containing *S. carnosus* and *S. xylosus* were lower during the first two days than found in other samples cured at 30℃ (figure 4). It suggests that the samples inoculated with *S. carnosus* and *S. xylosus* had a greater nitrate reducing activity than the sample inoculated with *M. varians* and the control. At 20℃, samples inoculated with all starter cultures had also presented a greater reducing activity than the control. However, the nitrate reducing activity of the sample inoculated with *S. xylosus* was slower than the sample inoculated with *M. varians* or *S. carnosus*.



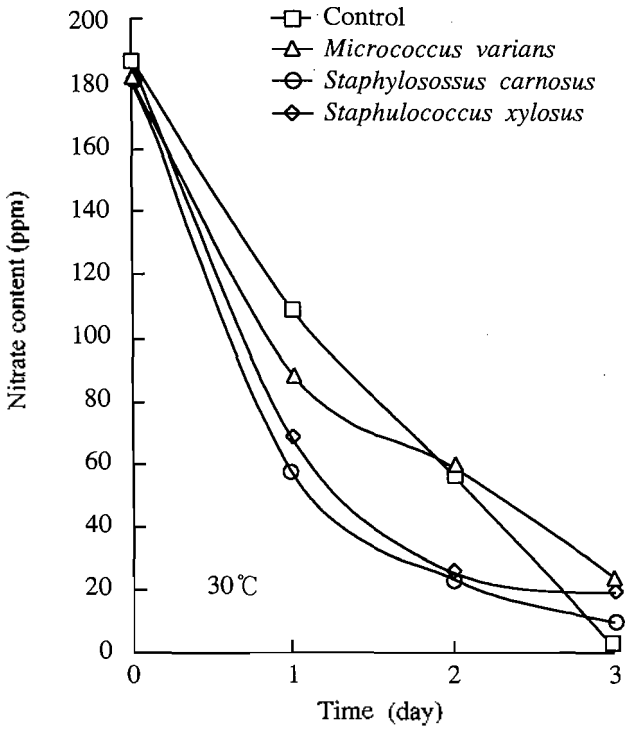


Figure 4. Nitrate residue concentrations of beaker sausage during curing period

The nitrite residues of all samples are shown in figure 5. Samples cured at 30℃ had better nitrite formation ability than samples cured at 20℃. After curing at 30℃ for 1 day, the nitrite residues of samples inoculated with *M. varians*, *S. carnosus* and *S. xylosus* were 86 ppm, 113 ppm and 117 ppm. Conversely, the samples contained *S. xylosus* and *S. carnosus* had a higher residual nitrite level than other samples when curing at 30℃. On the other hands, during curing at 20℃ for 1 day, the nitrite residues were 32 ppm, 85 ppm and 96 ppm for the sample inoculated with *M. varians*, *S. carnosus* and *S. xylosus*. Specially, sample inoculated with *S. xylosus* had increase in nitrite concentration after 2 days curing, the nitrite concentration rose to 106 ppm and then decreased after 3 day. Some studies have indicated that the nitrate reductase activity of Micrococcaceae started at 22-25℃ for the first 24 hr of curing (Hammes, 1986; Selgas et al., 1986). Table 2 showed that nitrate reducing and nitrite formation started at the first day. Temperature also showed the stimulatory effects on the conversion between nitrate and nitrite. Samples cured at 30℃ greatly increased the rate of nitrate reducing and nitrite formation than samples cured at 20℃. However, samples inoculated with starter cultures had higher nitrate reducing rate and nitrite formation rate. Over 50% of nitrate was converted by sample inoculated with starter culture and cured at 30℃ for 1 day. At the same time, the nitrite

Table 1. Formulation of beaker sausage

Ingredients	Treatment				
	Bank	Control	*Micrococcus varians*	*Staphylococcus carnosus*	*Staphylococcus xylosus*
Pork (kg)	1	1	1	1	1
Sodium chloride (g)	15	15	15	15	15
Sucrose (g)	20	20	20	20	20
Sodium nitrite (PPM)*	100	100	100	100	100
Sodium nitrate (PPM)*	-	150	150	150	150
M. varians (ml)**	-	-	50	-	-
Staphyl. carnosus (ml)**	-	-	-	50	-
Staphyl. xylosus (ml)**	-	-	-	-	50
Sterilized dist. water (ml)	50	50	-	-	-

* Based on meat weight; ** Equal to 10^7 CFU/g meat; - : Without addition.

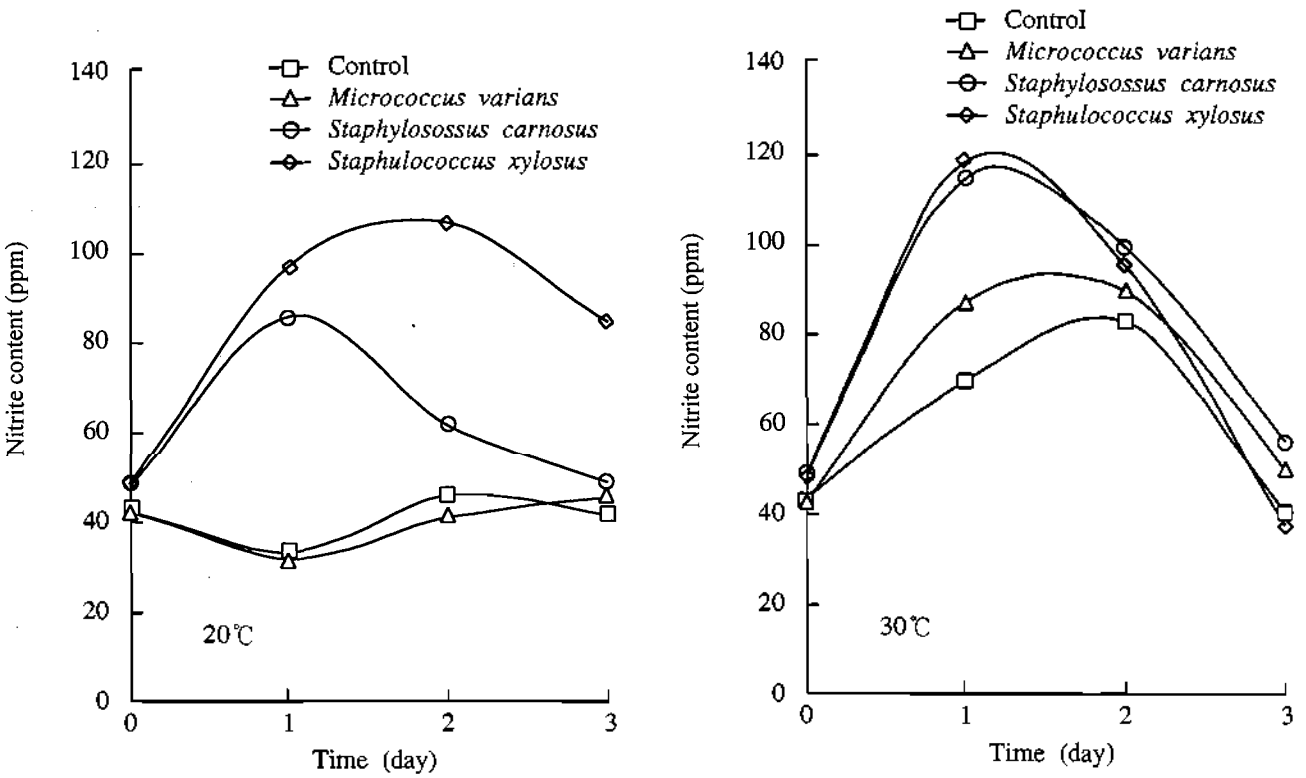


Figure 5. Nitrite residue concentrations of beaker sausage during curing period

ratio was rose to over 200%. However, a change in level can also be caused by chemicals and enzymes reaction in the muscles (Toth, 1983) as indicated by the results of the control samples shown in figure 5. Some researchers have reported a simultaneous oxidation of nitrite to nitrate in the presence of ferric ion (Fe^{3+}) and salt (Perez-Podriguez et al., 1996; Astiasaran et al., 1993). Nitrate might also originate from water, spices and raw materials (Wirth, 1986).

In this study it was observed that the same trends can be observed in nitrate reduction ability and the nitrosyl pigment formation as well the color retention. These results are in agreement with the report from Ory and Angelo (1977) who found that micrococci were capable of reducing various levels of nitrate with satisfactory formation of nitrosyl myoglobin which resulted in improvement of color. This research suggested that the nitrate reduction ability of Micrococcaceae, especially *Staphylococci caronsus* or *Staphylococcus xylosus* could play an important role in the quality of Chinese-style sausage. However, the utility of a starter, the technology of manufacturing and the optimization of the condition for processing Chinese-style sausages should be further investigated.

Table 2. Nitrate reducing and nitrite formation rate of beaker sausage during curing at 20℃ and 30℃

Treatment	Curing conditions (℃, day)					
	20℃			30℃		
	1	2	3	1	2	3
Residual nitrate ratio (%)*						
Control	87	58	10	58	30	2
Micrococcus varians	61	39	6	49	32	13
Staphylococcus carnosus	54	31	6	31	13	6
Staphylococcus xylosus	68	56	5	36	14	11
Residual nitrite ratio (%)*						
Control	78	106	97	160	190	92
Micrococcus varians	75	96	107	203	207	115
Staphylococcus carnosus	176	127	100	234	202	114
Staphylococcus xylosus	201	220	175	243	195	76

* Initial ratio of raw materials was defined as 100%. Residual nitrate ratio (%)=nitrate concentration of each batch/nitrate concentration of raw material; Residual nitrite ratio (%)=nitrite concentration of each batch/ nitrite concentration of raw material.

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