

## Color Stability of Chinese-Style Sausage Inoculated with *Staphylococcus Carnosus* and *Staphylococcus Xylosus*

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**ABSTRACT :** This study investigate the effects of starter cultures on the color stability of Chinese-style sausage. The samples were inoculated with  $10^7$  cfu g<sup>-1</sup> meat of either *Staphylococcus carnosus* or *Staphylococcus xylosus*. After mixing, curing at either 4°C or 20°C for 20 h and then drying at 50°C for 5 h, the samples were then either vacuum packed or hung at 4°C and 25°C (85% R. H.). The pH, nitrite content, nitrosyl pigment content, metmyoglobin and L-, a-, b- values were measured. The pH value still remained above 6.0 during storage. Nitrite residue of all samples decreased after storage at 25 °C for 7 days. The samples inoculated with *S. carnosus* and *S. xylosus* had higher nitrite content (20.9-34.7 ppm) than the control ( $p < 0.05$ ). Samples inoculated with *S. carnosus* and *S. xylosus* had higher nitrosyl pigment content and lower metmyoglobin content than those of the control. The L- and b- values of all samples decreased but the a- values increased with storage time. The result suggested that *S. carnosus* and *S. xylosus* starter cultures be used to improve color stability of Chinese-style sausage. (*Asian-Aust. J. Anim. Sci.* 2003. Vol 16, No. 4 : 570-574)

**Key Words :** Color, Metmyoglobin, Nitrite, Nitrosyl Pigment, Sausage, Staphylococci

### INTRODUCTION

Chinese-style sausage, a non-fermented product containing natural flora is very popular in Taiwan. Generally, it is processed with a short curing time of less than 2 days, stuffing and drying at 46-52°C for several hours. The shelf life of the products is only a month under cold storage in the supermarket, or a week when hanging at ambient temperature in the local market. However, one of the serious problems of this traditional product is the color stability. A bright red color sausage has been shown to be more acceptable to consumers than a grayish-red color one (Guo et al., 1992).

Sausages inoculated with starter cultures can be improved in quality. The most popular Micrococcaceae cultures are *Micrococcus varians*, *Staphylococcus carnosus* and *Staphylococcus xylosus* (Hammes and Knauf, 1994). They have catalase to destroy peroxide and can reduce nitrate to nitrite to improve color and flavor of sausages (Neubauer and Gotz, 1996; Geisen et al., 1992). In evaluating the use of starter cultures in the production of Chinese-style sausage, Huang and Lin (1995) selected *Lactobacillus plantarum* and a commercial culture (DS-66).

In a recent investigation, we have found that *S. carnosus* and *S. xylosus* have higher enzymatic activity when assessed by the reduction of metmyoglobin and nitrate, and by catalase and lipase activity (Guo et al., 2000). At the same time, we have also indicated that *S. carnosus* and *S. xylosus* can be the most suitable starter cultures for improving color formation during a modified aseptic curing environment for Chinese-style sausage (Guo et al., 2001). The aim of this study is to investigate the effects of *S. carnosus* and *S. xylosus* on nitrosyl pigment formation, nitrite content and color stability of Chinese-style sausage.

### MATERIALS AND METHODS

#### Preparation of starter culture

*Staphylococcus carnosus* (CCRC 12922) and *Staphylococcus xylosus* (CCRC 12930) were obtained from the Food Industry Research and Development Institute (FIRDI, Taiwan). Strains were inoculated on Mannitol Salt Agar (37°C, 24 h). Colonies were washed with sterilized distilled water and then added to sausage samples by  $10^7$  cfu<sup>-1</sup>g meat.

#### Preparation of sausage

The initial sausage mixture contained (W/W % based on weight of raw meat) ground lean pork from the ham (80%, 16 mm plate), pork back fat (0.8 cm<sup>3</sup> cubes, 20%). NaCl (1.5%), monosodium glutamate (0.5%), sucrose (2%), pepper (0.1%) and NaNO<sub>2</sub> (100 ppm). All ingredients were blended and mixed in a bowl and appropriate starter cultures ( $10^7$  cfu<sup>-1</sup>g) were added during mixing. The initial sausage was used as the control (without adding starter).

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Received November 5, 2001; Accepted March 11, 2002

The mixture was placed at either 4°C or 20°C in incubators for 20 h and then stuffed into commercial casings (30 mm.). The sausages were dried at 50°C for 5 h, then stored either by hanging in an incubator at 25°C (R.H. 85%) for 7 days or in a vacuum packed storage at 4°C for 28 days.

**Measurement of pH value**

Ten grams of sausage samples were homogenized (10,000 rpm, 1 min) with 90 ml of distilled water and the pH was measured by a pH meter (TX-1 Suntex, Taiwan).

**Nitrite assay**

Five grams of sample were homogenized with 80 ml of 80°C distilled water which were then mixed at 80°C in a water bath for 30 min. After filtering ( Whatman No. 1 filter paper ) and allowed color with Griess solution for 30 min. Nitrite content was calculated for its Optical Density (O.D.) at 540 nm (Ockerman, 1981).

**Nitrosyl pigment assay**

Ten grams of samples were extracted with 40 ml of acetone and 2 ml of distilled water. The homogenate was filtered and nitrosyl pigment was calculated for the O.D. at 540 nm using a spectrophotometer (U-3210, Hitachi, Japan) (Ockerman, 1981).

**Metmyoglobin assay**

Metmyoglobin was prepared and measured according to the method of Trout (1989). Ten grams of sample were extracted (at 0°C) with 0.04 M phosphate buffer (pH 6.8). After homogenization the product was centrifuged at 10,000 rpm for 1 min, homogenate was centrifuged and filtered with Whatman No. 1 filter paper. Absorbance of the filtrate was measured at 525, 572 and 700 nm using a Hitachi U-3210 spectrophotometer, and percentage metmyoglobin concentration was calculated as follow:

$$\text{Metmyoglobin (\%)} = \{ 1.395 - [(A_{572} - A_{700}) / (A_{525} - A_{700})] \} * 100$$

**Color determination**

The color of sausage samples was expressed by the Hunter L-, a-, and b- values using the Handy Colorimeter (NR-300, Nippon Denshoku, Japan).

**Statistical analysis**

Data were analyzed using a statistics software package (SAS, 1995). The ANOVA system was used to test the significance of treatment effects, when significant (p<0.05) overall differences were found, differences between individual means were then assessed by Duncan's multiple range test.

**RESULTS AND DISCUSSION**

**pH value**

pH value of samples only decreased from 6.36-6.41 to 5.66-5.96 even stored at 25°C for 7 days (Table 1). The decrease of 0.5-0.7 units of pH showed that the sugar in the Chinese-style sausage was not completely fermented. When samples were stored at 4°C, the pH value remained above 6.0 (Table 1).

Researchers in previous studies reported that Chinese-style sausages were dry, or rarely semi-dry products, and that the pH was relatively high between 5.8-6.2 (Savic et al., 1988). The pH of this product decreased remarkably slow when manufactured by using traditional processing (Chen et al., 1997). This indicated that bacteria did not degrade sugar to lactic acid in Chinese-style sausage because of shorter fermentation or non-fermentation. Simultaneously in our previous study (Guo and Chen, 1991) we found that Micrococcaceae was the predominant flora in Chinese-style sausage. Thus, such an environment was not only advantageous for the growth of Micrococcaceae, which is less tolerant of low pH (Lucke, 1986), but it also increased the activity of nitrate reductase and increased the extent of metmyoglobin reduction (Giddings, 1974; Puolane et al., 1977).

**Nitrite content**

After drying, the nitrite content was measured between 85.5- 95.0 ppm and 86.3-91.6 ppm when the samples were cured at 25°C and 4°C, respectively (Table 2). Samples inoculated with starter culture were higher in nitrite residue than the control samples after drying and storage. All samples rapidly decreased in nitrite content with increasing storage times, but the samples with *S. carnosus* and *S. xylosus* had significant higher (p<0.05) residual nitrite levels than the control sample during storage.

**Table 1.** Changes in pH of Chinese-style sausage inoculated with *Staphylococcus carnosus* and *Staphylococcus xylosus* during storage at 4°C and 25°C

Treatments	After curing for 20 h	After drying	Storage temperature and time	
			25°C, 7 day	4°C, 28 day
<b>20°C curing</b>				
Control	6.39	6.46	5.68	6.65
<i>Staphylococcus carnosus</i>	6.34	6.48	5.96	6.57
<i>Staphylococcus xylosus</i>	6.36	6.53	5.74	6.48
<b>4°C curing</b>				
Control	6.44	6.51	5.73	6.17
<i>Staphylococcus carnosus</i>	6.41	6.51	5.69	6.54
<i>Staphylococcus xylosus</i>	6.41	6.55	5.71	6.29

There are no significant differences between means in the same column (p<0.05).

**Table 2.** Changes in nitrite content (ppm) of Chinese-style sausage inoculated with *Staphylococcus carnosus* and *Staphylococcus xylosus* during storage at 4°C and 25°C

Treatments	After curing for 20 h	After drying	Storage temperature and time	
			25 °C	4 °C
			7 day	28 day
<b>20°C curing</b>				
Control	87.8 <sup>a</sup>	85.5 <sup>bc</sup>	11.4 <sup>c</sup>	21.3 <sup>c</sup>
<i>Staphylococcus carnosus</i>	85.3 <sup>a</sup>	80.5 <sup>c</sup>	20.9 <sup>c</sup>	46.3 <sup>a</sup>
<i>Staphylococcus xylosus</i>	86.9 <sup>a</sup>	95.0 <sup>a</sup>	35.7 <sup>a</sup>	48.1 <sup>a</sup>
<b>4°C curing</b>				
Control	87.0 <sup>a</sup>	86.3 <sup>ab</sup>	16.0 <sup>c</sup>	26.7 <sup>b</sup>
<i>Staphylococcus carnosus</i>	84.3 <sup>a</sup>	89.1 <sup>ab</sup>	29.9 <sup>b</sup>	44.4 <sup>a</sup>
<i>Staphylococcus xylosus</i>	88.1 <sup>a</sup>	91.6 <sup>a</sup>	34.7 <sup>ab</sup>	44.5 <sup>a</sup>

<sup>a,b,c</sup> Means within the same columns with different superscripts, but without a common superscript letters within the same column are significantly different ( $p < 0.05$ ).

There were some different results when the current processes were compared with traditional processes. Chen and Huang (1975) indicated that when samples dried at 60 and 50°C and stored for two days, nitrite content decreased from 40 and 10 ppm. In this study, results are in agreement with the reports from other researchers (Perez-Podriguez et al., 1996; Astiasaran et al., 1993; Wirth, 1986), which indicate that some nitrate originated from the formula might have been reduced by Staphylococci after drying. Since bacterial counts of Micrococcaceae of Chinese-style sausage were measured between 6-7 cfu g<sup>-1</sup>, it may suggest that the bacteria were enough to reduce nitrate to form nitrite as reported by Sanz et al. (1997).

### Nitrosyl pigment and metmyoglobin

Levels of 8.1-8.5 ppm and 6.9-7.0 ppm of nitrosyl pigment were found in the samples cured at 20°C and 4°C

**Table 3.** Changes in Nitrosyl pigment (ppm) of Chinese-style sausage inoculated with *Staphylococcus carnosus* and *Staphylococcus xylosus* during storage at 4°C and 25°C

Treatments	After curing for 20 h	After drying	Storage temperature and time	
			25 °C	4 °C
			7 day	28 day
<b>20°C curing</b>				
Control	8.1 <sup>a</sup>	15.3 <sup>b</sup>	13.6 <sup>c</sup>	14.5 <sup>a</sup>
<i>Staphylococcus carnosus</i>	8.1 <sup>a</sup>	17.6 <sup>a</sup>	15.8 <sup>a</sup>	14.6 <sup>a</sup>
<i>Staphylococcus xylosus</i>	8.5 <sup>a</sup>	17.7 <sup>a</sup>	15.3 <sup>ab</sup>	15.1 <sup>a</sup>
<b>4°C curing</b>				
Control	6.9 <sup>b</sup>	11.5 <sup>c</sup>	12.2 <sup>c</sup>	9.2 <sup>b</sup>
<i>Staphylococcus carnosus</i>	6.6 <sup>b</sup>	16.1 <sup>ab</sup>	13.6 <sup>c</sup>	14.9 <sup>a</sup>
<i>Staphylococcus xylosus</i>	7.0 <sup>b</sup>	15.6 <sup>b</sup>	13.9 <sup>c</sup>	15.2 <sup>a</sup>

<sup>a,b,c</sup> Means within the same columns with different superscripts, but without a common superscript letters within the same column are significantly different ( $p < 0.05$ ).

for 20 hrs, respectively (Table 3). Nevertheless the most of the myoglobin was remarkably converted to nitrosyl myoglobin after drying. Samples inoculated with *S. carnosus* or *S. xylosus* and stored at 25°C had higher ( $p < 0.05$ ) nitrosyl pigment levels than the control. It is possible that the control sample naturally fermented at 4°C under anaerobic conditions, as the lactic acid bacteria were the predominant flora in the late period of lowering ability to form nitrosyl pigment.

Residual metmyoglobin levels in Table 4 showed that samples inoculated with *S. xylosus* had a higher ability to reduce metmyoglobin. After drying, the metmyoglobin of samples cured at 25°C was lower than the sample cured at 4°C. Moreover, when the sample were hung at 25°C for 7 days, the value of metmyoglobin remained stable but when the sample stored at 4°C for 28 days the value increased.

These results showed that the main factors affect color development and color stability were temperature (heating) and starter culture inoculation. Reaction from endogenous system also played an important role (Giddings, 1974). However, a report from Vosgen (1992) suggested that 70-80% of muscle myoglobin reacted with nitrogen oxide and gave a reddish color. As reported by Livingston and Brown (1981), denaturation of myoglobin mainly occurred when heated at above 80°C or by when the pH decreased to below 5. In particular, Chinese-style sausage is dried at below 55°C for several hours. Therefore, myoglobin and nitrosyl myoglobin could be oxidized to metmyoglobin and nitrosyl metmyoglobin in order to form the desired greyish-brown color. In comparison, Faustman et al. (1990) indicated that metmyoglobin might be converted back to a physiologically active form by endogenous reduction. It has been reported that microorganisms belonging to genus *Staphylococcus* can convert metmyoglobin to red myoglobin derivatives

**Table 4.** Changes in metmyoglobin content (%) of Chinese-style sausage inoculated with *Staphylococcus carnosus* and *Staphylococcus xylosus* during storage at 4°C and 25°C

Treatments	After curing for 20 h	After drying	Storage temperature and time	
			25 °C	4 °C
			7 day	28 day
<b>20°C curing</b>				
Control	80.3 <sup>ab</sup>	78.7 <sup>b</sup>	78.6 <sup>bc</sup>	78.9 <sup>b</sup>
<i>Staphylococcus carnosus</i>	80.0 <sup>ab</sup>	77.2 <sup>c</sup>	79.0 <sup>abc</sup>	79.3 <sup>ab</sup>
<i>Staphylococcus xylosus</i>	78.8 <sup>b</sup>	76.2 <sup>c</sup>	78.0 <sup>c</sup>	78.3 <sup>b</sup>
<b>4°C curing</b>				
Control	81.9 <sup>a</sup>	80.6 <sup>a</sup>	81.5 <sup>a</sup>	81.3 <sup>a</sup>
<i>Staphylococcus carnosus</i>	82.0 <sup>a</sup>	79.2 <sup>ab</sup>	80.8 <sup>ab</sup>	80.8 <sup>a</sup>
<i>Staphylococcus xylosus</i>	80.0 <sup>ab</sup>	79.3 <sup>ab</sup>	80.6 <sup>abc</sup>	80.9 <sup>a</sup>

<sup>a,b,c</sup> Means within the same columns with different superscripts, but without a common superscript letters within the same column are significantly different ( $p < 0.05$ ).

**Table 5.** Changes in Hunter color parameter of Chinese-style sausage inoculated with *Staphylococcus carnosus* and *Staphylococcus xylosus* during storage at 4°C and 25°C

Treatments	After drying			Storage temperature and time					
	L	a	b	25°C, 7 day			4°C, 28 day		
Hunter color parameter 20°C curing				L	A	b	L	a	b
Control	36.3 <sup>bc</sup>	11.9 <sup>b</sup>	9.2 <sup>a</sup>	29.0 <sup>ab</sup>	13.8 <sup>a</sup>	8.4 <sup>a</sup>	50.3 <sup>b</sup>	12.2 <sup>b</sup>	8.8 <sup>b</sup>
<i>Staphylococcus carnosus</i>	36.9 <sup>ab</sup>	12.3 <sup>ab</sup>	9.8 <sup>d</sup>	29.8 <sup>a</sup>	13.9 <sup>a</sup>	8.3 <sup>a</sup>	51.0 <sup>b</sup>	13.1 <sup>ab</sup>	9.4 <sup>a</sup>
<i>Staphylococcus xylosus</i>	35.9 <sup>c</sup>	13.1 <sup>a</sup>	9.4 <sup>d</sup>	28.3 <sup>ab</sup>	14.5 <sup>a</sup>	8.3 <sup>a</sup>	51.8 <sup>ab</sup>	14.2 <sup>a</sup>	8.7 <sup>b</sup>
4°C curing									
Control	37.4 <sup>abc</sup>	12.0 <sup>b</sup>	9.1 <sup>a</sup>	29.9 <sup>a</sup>	13.8 <sup>a</sup>	8.5 <sup>a</sup>	51.5 <sup>ab</sup>	12.7 <sup>b</sup>	9.2 <sup>ab</sup>
<i>Staphylococcus carnosus</i>	38.3 <sup>a</sup>	12.5 <sup>ab</sup>	9.9 <sup>a</sup>	28.6 <sup>ab</sup>	14.6 <sup>a</sup>	8.6 <sup>a</sup>	50.9 <sup>b</sup>	13.5 <sup>ab</sup>	8.9 <sup>b</sup>
<i>Staphylococcus xylosus</i>	37.9 <sup>ab</sup>	13.2 <sup>a</sup>	9.8 <sup>a</sup>	27.9 <sup>b</sup>	14.3 <sup>a</sup>	8.3 <sup>a</sup>	52.8 <sup>a</sup>	13.0 <sup>ab</sup>	9.0 <sup>ab</sup>

<sup>a,b,c</sup> Means within the same columns with different superscripts, but without a common superscript letters within the same column are significantly different (p<0.05).

(Morita et al., 1994) and inhibit discoloration of meat products (Morita et al., 1997), possibly due to NADH-cytochrome b<sub>5</sub> reductase systems, including metmyoglobin reductase and nitrate reductase (Arihara et al., 1995; Lillo and Peter, 1992). Thus, some of *Staphylococcus* such as *S. carnosus* or *S. xylosus* may be useful in the production of Chinese-style sausage.

**Color**

Table 5 showed that all the samples cured at 20°C had lower L- values than the samples cured at 4°C, and that L-value decreased after being hung at 25°C. These results indicated that the sausage changed to a darker colour during storage. However, the sample stored under vacuum packed at 4°C for 28 days increased in L- value. The samples inoculated with *S. carnosus* and *S. xylosus* had significantly higher a- value than the control. All of the samples increased in a- value after storage at 25°C for 7 days. Value of a- for samples stored under vacuum packed at 4°C increased slowly. The samples inoculated with starter cultures were also higher in b- value than the control during hanging storage due to the production of yellow pigments by the two strains. All of the samples decreased in b- value after 7 days at 25°C or 28 days at 4°C.

**CONCLUSION**

The sample inoculated with *S. carnosus* and *S. xylosus* had higher residual nitrite content, higher stability of nitrosylpigment and the tower metmyoglobin content and they also gave the a product with a brighter red color. It is concluded that Chinese-style sausage inoculated with *S. carnosus* and *S. xylosus* could enhance color stability during manufacturing and storage.

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