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The effects of mixed starter cultures on the quality of dry fermented Chinese-style sausages

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ABSTRACT

Three different batches of dry fermented Chinese-style sausages; CHS-LC, CHS-SX and CHS-SL were prepared separately inoculating with *Lactobacillus casei* subspp *casei*-1.001, *Staphylococcus xylosus*-12 and a mixture of these two starters in equal proportion. The water activity and pH ranged from 0.795 to 0.826 and 4.10 to 5.26 respectively with no significant difference in weight losses in final products. Likewise, residual nitrite and volatile basic nitrogen ranged from 1.99 to 3.52 ppm and 15.17 to 18.56mg/100g respectively. Moreover, the non protein nitrogen (NPN), alpha amino acids (ANN) as well as red color were increased in batch CHS-SL. However, the texture profile analysis showed no changes (P>0.05). Lactic acid bacteria were predominated microbe after fermentation in all batches, which showed good inhibitory effects to the growth of *Micrococcaceae, Enterobacteriaceae* and Yeast and Molds during ripening. Sensory evaluation result showed that the batch CHS-SL was superior (P<0.05) in overall quality attributes. Therefore, mixed cultures of *L. casei* subspp *casei*-1.001 and *S. xylosus*-12 would be the most appropriate meat starter culture for producing dry fermented Chinese-style sausage.

Keywords: Dry fermented Chinese -style sausage; *Lactobacillus casei; Staphylococcus xylosus*; Mixed starter culture; Quality attributes

INTRODUCTION

Semi-dry or natural fermented sausage is one of the popular Chinese-style sausages. Where pork is the main meat source , sugar, rice wine and some spices are common ingredients used. It is made out of ground meat with a larger particle size than other kinds of sausages (Yu and Chou,1997).Traditionally, the processing technique involves mixing of ground pork with curing ingredients ,and cured at 2-5^{0}C for 1-2 days. Finally, stuffed into a pork casing and naturally dried in sun or electric heater at 50 to 55^{0}C for 8 to 24 hr to get final yield of 62 to 73% (Huang and Lin, 1993; Lin and Chao , 2001).

The use of acidifying organisms, especially lactic acid bacteria (LAB) and nitrate–reducing *Micrococci* negative

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Staphylococci have been reported as main meat starter culture for dry fermented sausages (Hugas, 1998; Marchesini *et al*. 1992, González and Díez, 2002), though their use in Chinese-style sausage is very limited. However, the quality of Chinese-style sausage could be improved either by adding lactic acid bacteria (Huang and Lin, 1993) or *Staphylococci* such as *S. xylosus* (Guo *et al*. 2000). Lactic acid bacteria can produce mainly lactic acid and bacteriocin. Lactic acid lowers the pH of meat and bacteriocin acts against the growth of pathogens as well as other spoilage bacteria, hence stabilize the product biologically (Con and Gökalp, 2000; Yin and Jiang, 2001; Fadda *et al*., 2002).

Furthermore, the use of coagulase negative *Staphylococci* as starter in dry fermented sausage would not only produce important volatile components (Montel *et al*. 1996; Stahnke, 1999; Søndergaard and Stahnke, 2002; Olesen *et al.* 2004) but it could also remove the hydrogen peroxide produced by *Lactobacilli*, which can cause colour problem in products (Marchesini *et al*. 1992; Hugas and Monfort, 1997).That is why the mixed culture have been widely used in manufacturing of dry fermented sausages.

To date, hardly any work has been published on the use of mixed culture (i.e. *Lactobacilli* and *Staphylococci*) in dry fermented Chinese-style sausages. Therefore, aim of this work was to determine the physico-chemical, microbiological as well as sensory quality characteristics of dry fermented Chinese-style sausages inoculated with mixed starter cultures.

MATERIALS AND METHODS

Culture collection and preparation

Lactobacillus casei.subspp.casei-1.001 (From China general microbiological culture collection center, Beijing) and *Staphylococcus xylosus* -12 (From Technological Centre of Shunghui Group, Henan, China) were used as meat starter cultures for dry fermented Chinese-style sausages. Sausage samples with single starter culture served as the control. The *L. casei.subspp.casei*-1.001 was sub cultured twice in MRS broth at 30^0C for 2 days and finally cells were harvested by centrifugation (10000g for 15min at 4^0C), washed twice with 20mMl l^{-1} phosphate buffer , pH 7.0 and finally resuspended in same buffer (20% of the initial volume). Similarly, *Staphylococcus xylosus*-12 was sub cultured in nutrient broth at 30^0C for 3days and cells were harvested (2000g for 15min at 4^0C), washed twice with saline water (8.5g NaCl/L) then resuspended in the same saline water (20% of the initial volume). The harvested cells were immediately inoculated in sausage batter in the range of 10^7cfu/g by adjusting optical density at 680 nm for lactic acid bacteria and 640nm for *S. xylosus* -12.

Product formulation

The dry fermented Chinese style sausage was prepared according to the recipe given in Table 1.

Table 1- Curing ingredients and starter cultures used for the production of dry fermented Chinese-style sausages

Ingredients	g/100g meat
Sugar	8.0
Salt	2.0
MSG powder	0.5
Sodium nitrite	0.012
Sodium erythorbate	0.05
Potassium sorbate	0.2
Sodium tripolyphosphate	0.2
Rice wine	1.0
Chilled water	10
Five spices powder	0.3
White pepper powder	0.1
S. xylosus-12	~$8.7x10^6$ /g
L. casei.subspp.*casei*-1.001	~$9.7\ x10^6$ /g
S. xylosus + L. casei subspp.*casei*-1.001	1:1

Manufacturing of sausages

Fresh boneless pork ham, back fat and other curing ingredients were purchased from local supermarkets. Heavy connective tissues and external fat were trimmed off and frozen below -10^0C overnight. The lean and back fat were partially thawed before grinding through a 9.5mm plate meat mincer. The minced meat portion was mixed with curing ingredients as given in Table 1. Altogether, three different batches (each batch of 2kgs) were prepared, where batch CHS-SX was inoculated with *S. xylosus*-12; batch CHS-LC with *L. casei.subspp.casei*-1.001 and mixed culture batch CHS-SL was with *S. xylosus*-12 and *L. casei.subspp.casei*-1.001 in equal proportion (Table 1). After curing at 4^0C for 24 hrs , stuffed into edible collagen casing (d ≈ 3 cm, Nalo Faser, Kalle GmbH & Co. KG, Germany) and linked manually (each link about 10-12cm long and 50-55 g in weight). Finally, raw sausages were kept inside a laboratory ripening cabinet (Zhujian, LRH-150-SII, China) and programmed as follows: fermentation at 22±1^0C and 90-95%RH for 3days, followed by first ripening at 15±1^0C and 80-85% RH for one week and final ripening at 12±1^0C and 70-75% RH for two weeks.

Sampling

Five links of sausage samples from each batch were taken randomly at following processing stages: before stuffing (0 days); after fermentation (3rd day); after one week of ripening (10th day) and final products (24th day).The samples were immediately analyzed.

Physico-chemical analysis

For the weight losses, three similar links of sausages from each batch were weighed just before placing in the ripening cabinet i.e. at 0 day. The same strings of sausages were reweighed on the 3^{rd}, 10^{th}, and 24^{th} day. The difference in weight was expressed as percentage of weight loss based on initial weight.

Moisture content, crude protein and crude fat content were determined following the AOAC (1995) and salt content was determined by (Rangana 1991); pH (Wang 2000); Volatile basic nitrogen (VBN) according to Yin and Jiang 2001 and Pearson

(1968); Residual Nitrite (AOAC 1995); Free Amino Nitrogen as α- amino nitrogen (AAN) (AOAC 1980); Non Protein Nitrogen (NPN) (Dierick *et al*., 1974); Texture profile analysis according to Bourne (1978) and Lin and Chao (2001); Colorimetric analysis (Hunter *L*, *a*, *b* values) by using colorimeter (Model TC–PII G, Beijing Optical Instruments Factory);Water activity was noted using a_w meter (Rotronic Hygroskop DT, Swiss made) after equilibration at 25^0C.

Microbiological analysis

Lactic acid bacteria (LAB) in MRS agar incubated at 30^0C for 2 days; *Micrococaceae* in MS agar (MSA) incubated at 30^0C for 2 days (González and Díez 2002); *Enterobacteriaceae* count in Violet Red Bile Agar (VRBD) incubated at 37^0C for one day (Babji and Murthy 1999) and Yeast and molds count in Potato Dextrose Agar (PDA) according to (Refai, 1979).

Sensory evaluation

Experienced 15-member panels were used to evaluate for appearance, red color, flavor, sourness, sweetness, saltiness, rancidity, hardness and overall acceptability of dry fermented Chinese -style sausage. A 0-6 rating scale was used for each attribute, where 0 = very low intensity and 6 = very high intensity (Moretti *et al*., 2004).A preparatory session was held just before testing. Assessments were performed under natural light. Potable water was provided prior to taste and between interval of samples to clean their palate.

Statistical analysis

One way analysis of variance (ANOVA) was applied for statistical data analysis. For significant differences (P<0.05), mean separation were accomplished with pool means by using Duncan's multiple range test. (Glover and Mitchell, 2001). Pearson coefficient of correlation was obtained by

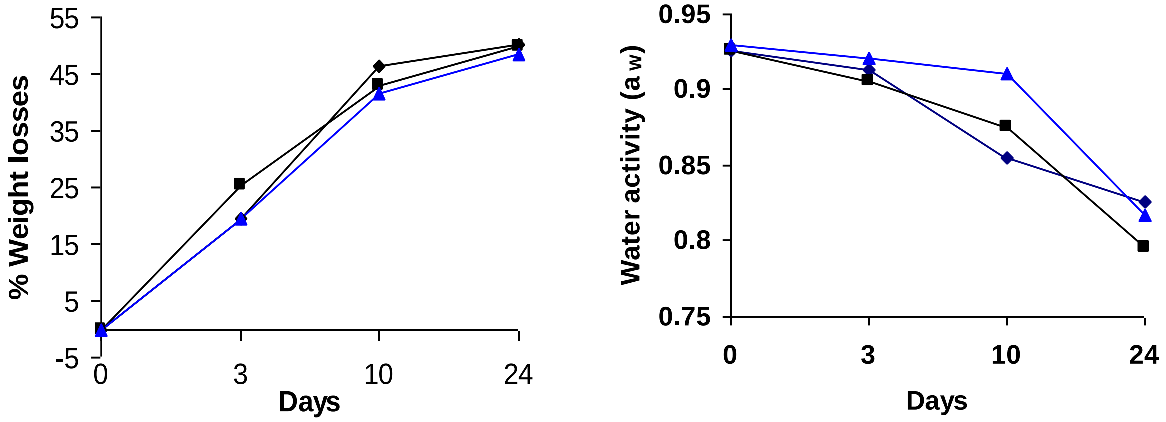


Fig 1- Effects of mixed starter cultures on the weight losses and water activity during the fermentation and ripening of batches: -◆-◆- (CHS-LC); -■-■-(CHS-SX) and -▲-▲-(CHS-SL).

using SPSS 11.5 Inc. (2002). For each treatment, at least 3 replicates were performed and the pooled means were taken for result.

RESULTS AND DISCUSSION

Physico-chemical parameters

The weight losses and changes in water activity of sausages during fermentation and ripening are shown in Fig 1. A considerable weight loss (48.35 to 50.12%) has been found (Fig 1A) during ripening of the dry fermented Chinese-style sausage, however the weight losses were not significant (P>0.05) to each others.

Similarly, results of physico-chemical analysis are given in Table 2. The moisture content of final product was in the range of 24.8 to 26.85 %. Likewise, Protein content varied from 30.12 to 33.88%, fat content from 27.32 to 28.19% and the salt content from 3.04 to 3.42 with no significant difference in case of fat and salt. In parallel, a_w decreased from initial values of around 0.93 to final range 0.795 to 0.826. These finding are in general agreement with salami and other dry fermented sausages (Dellaglio *et al*., 1996; Moretti *et al*., 2004). The variations in treatments were attributed to lack of uniformity in mixing of coarse ground meat particles and fat with curing mixtures to some extent.

Table 2- Physico-chemical analysis of dry fermented Chinese-style sausages

Parameters	Batches		
	CHS-LC	CHS-SX	CHS-SL
Moisture %	26.07^{ab} ± 0.18	24.80^{a} ± 1.16	26.85^{b} ± 0.22
Protein %	31.03^{a} ± 0.66	33.88^{b} ± 1.14	30.12^{a} ± 0.78
Fat %	27.32^{a} ± 0.66	28.19^{a} ± 0.95	27.53^{a} ± 1.28
Salt %	3.42^{a} ± 0.12	3.04^{a} ± 0.11	3.18^{a} ± 0.20
pH	4.10^{a} ± 0.10	5.26^{b} ± 0.03	4.95^{c} ± 0.03
VBN (mg/100g)	15.17^{a} ± 0.61	17.53^{b} ± 0.75	18.56^{b} ± 1.16

Means in the same row having different superscripts are significantly different (P<0.05)
Mean ± Standard deviation; all results are expressed on a wet weight basis.

The batch CHS-LC (inoculated with *L.casei*.subspp.*casei*-1.001) showed the lowest (P<0.05) pH value and volatile basic nitrogen (VBN) in final product, mean while batch CHS-SX (inoculated with *S. xylosus* -12) showed higher (P<0.05) pH value and VBN in final product, though the VBN value was insignificant (P>0.05) to treatment CHS-SL (Table 2).Our result also agreed with those of Yin and Jiang, 2001; Yin et al., 2002., who have reported that the LAB could subsequently inhibit the accumulation of VBN by producing lactic acid and bacteriocin that could neutralize the volatile basic nitrogen during fermentation and ripening of sausages.

The initial level of residual sodium nitrites ranged from 79.37 to 84.58ppm, which was sharply decreased after fermentation to the level below 4 ppm in final products (Table 3).

Table 3- Effect of mixed starter cultures on residual sodium nitrite in dry fermented Chinese-style sausages

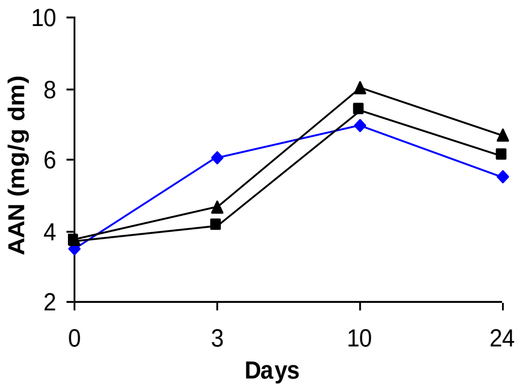
Days	Batches		
	CHS-LC	CHS-SX	CHS-SL
	$84.58^{b} \pm 0.35$	$79.37^{a} \pm 0.62$	$83.38^{b} \pm 1.74$
3	$17.76^{a} \pm 1.04$	$24.23^{b} \pm 1.15$	$19.99^{c} \pm 0.17$
10	$3.28^{a} \pm 0.16$	$4.38^{b} \pm 0.47$	$10.84^{c} \pm 0.70$
24	$1.99^{a} \pm 0.37$	$3.52^{b} \pm 0.35$	$3.46^{b} \pm 0.53$

Means with different superscripts within same row are significantly different ($P<0.05$) Mean value of sodium nitrite in ppm ± standard deviation

The batch CHS-LC inoculated lactic acid bacteria had the lowest (P<0.05) value for nitrite, while remaining two batches were insignificant (P>0.05) to each other. The residual sodium nitrite level in semi-dry fermented Chinese- style sausage has been reported below 10ppm (Huang and Lin 1993), while it has been also reported in trace level in dry fermented salami (Marchesini *et al.*, 1992) and Chinese fermented fish sausage inoculated with lactic acid bacteria (Yin and Jiang, 2001). Sodium nitrite can produce several carcinogenic nitrosamines (NAS 1981). Therefore, higher concentration level of residual nitrite could affect the nitrosamine level in final products (Fiddler *et al.*, 1996; Sen *et al.*, 1974). In this study, our result showed a trace or negligible amount of residual nitrite in final products.

Proteolyitc related parameters such as AAN and NPN during fermentation and ripening of dry fermented Chinese-style sausage are shown in Fig 2.

Initially, the AAN values in sausages varied from 3.51 to 3.77 mg/g (dry mass) and NPN from 5.18 to 5.96mg/g (dry mass) (P>0.05) in both cases. The AAN and NPN values in samples were normally



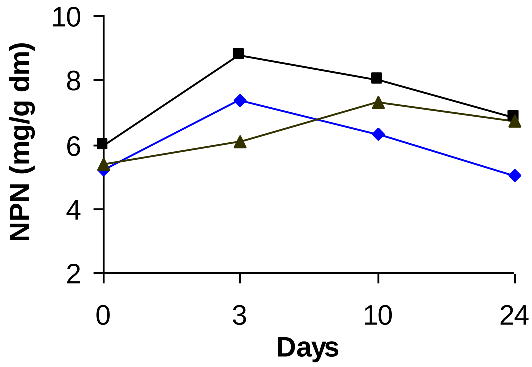


Fig 2- Effects of mixed starter cultures on the formation of free amino acid (AAN) and non protein nitrogen (NPN) during fermentation and ripening of batches: -▲-▲-(CHS-SL); -◆-◆-(CHS-LC); -■-■- (CHS-SX).

increased with ripening time reaching a maximum in first week then gradually decreased to a range of 5.54 to 6.71 mg/g (dry mass) and 5.03 to 6.82mg/g (dry mass) in final products respectively (Fig 2A and 2B).

Mixed culture batch (CHS-SL) showed significantly higher (P<0.05) AAN and NPN values but NPN was not significantly different (P>0.05). Our result agreed with Bover-Cid *et al*. (2000), who have reported that mixed culture of *S.xylosus* and LAB yielded higher amount of total free amino acids as well as NPN values in dry fermented sausage. Moreover, strong proteolytic activity of *S. xylosus* in dry fermented sausage has been reported that could increase the proteolytic related parameters (Bover- Cid *et al*. 1999).

Effect of mixed culture on the Hunter *L*, *a* , *b* values of dry fermented Chinese-style sausage is shown in Table 4.

Table 4- Effect of mixed starters culture on the Hunter *L, a, b* of dry fermented Chinese- style sausages

Color value	Batches		
	CHS-LC	CHS-SX	CHS-SL
L	$35.09^{b} \pm 2.83$	$27.84^{a} \pm 2.17$	$35.50^{b} \pm 1.04$
a	$8.38^{b} \pm 0.94$	$9.25^{b} \pm 0.81$	$13.56^{a} \pm 1.48$
b	$4.73^{ab} \pm 1.08$	$6.27^{b} \pm 0.68$	$3.03^{a} \pm 1.20$

Means having different superscripts within the same row are significantly different (*P*<0.05). Mean of triplicates ± Standard deviation

Batch (CHS-SL) added with mixed culture starter showed the highest (P<0.05) *a* value. It is also evident from the sensory result, where the sample from the same batch (CHS-SL) had significantly higher red color score ((P<0.05).

The result obtained for texture profile analysis of dry fermented Chinese-style sausage is given in Table 5.

The values for springiness and cohesiveness in different samples were not significant (P>0.05). The batch CHS-SL added with mixed culture showed the lowest (P<0.05) value for hardness, chewiness and gumminess but the values were not significantly different with the batch CHS-SX, mean while the treatment (CHS-LC) showed the highest (P<0.05) values to the same. A significant correlation (r = 0.705, P<0.05) was found between hardness and weight losses during ripening. Similar results have been reported by Bloukas *et al*., (1997). A higher negative correlation (r = -0.643, P<0.05) has been found between pH and hardness in dry sausage. Additionally, fat also showed negative correlation with the TPA parameters. In contrast, salt showed higher positive correlation with TPA parameters. Our results agreed with the results of other authors, who have been reported similar correlations between fat, pH, protein and salt with TPA parameters in sausages (Gimeno *et al*., 2000; Matulis *et al*., 1995).The higher water losses during ripening could influence the texture of sausage. The variations in values could be attributed to coarse meat particle sizes that are used in Chinese–style sausage. This type of inconsistency also would be related with the degree of proteolysis by predominated microbes in dry fermented sausages (Herranz *et al.* 2003).

Fig 3(A) shows the changes in total LAB counts during fermentation and ripening of dry fermented Chinese-style sausage.

Table 5- Effects of mixed starter cultures on textural characteristics of dry fermented Chinese-style sausages (Mean of triplicates and standard deviation in parentheses)

Parameters	Batches		
	CHS-LC	CHS-SX	CHS-SL
Hardness (g)	13391.44^{b} (708.95)	11195.61^{a} (214.76)	9483.50^{a} (1571.67)
Springiness (mm)	0.83^{a} (0.07)	0.897^{a} (0.12)	0.879^{a} (0.03)
Cohesiveness (Ratio)	0.53^{a} (0.032)	0.483^{a} (0.03)	0.459^{a} (0.04)
Gumminess (g)	7122.85^{b} (262.81)	5402.31^{a} (993.58)	4380.79^{a} (170.99)
Chewiness (g)	5909.76^{b} (75.34)	4865.53^{ab} (634.79)	3857.90^{a} (985.51)

Means with different superscripts within the same row are significantly different ($P<0.05$)

In all batches, the total plate counts were increased to a level 10^{-8} after fermentation and counts were not changed (P>0.05). The initial counts of *Micrococcaceae* in samples were ranged at 10^{4} to 10^{7} (Fig. 3B).The counts were obviously higher in the samples used S.xylosus-12 as culture. The *Micrococcaceae* counts for batch CHS -LS were significantly increased (P<0.05) to a level of 10^{8} after fermentation. In contrast, the counts were rapidly decreased (P<0.05) in treatment CHS-LC after fermentation stage. There are several authors who have reported that acidification was the main cause of inhibition to *Micrococcaceae* in dry fermented sausage (Lizaso *et al*. 1999). In this study, the pH value for treatment CHS-LC has been found to be the lowest (Table 2), which might be the reason for inhibition of *Micrococcaceae* in batch CHS-LC inoculated with *L.casei*.subspp.*casei*-1.001. The initial counts for *Enterobacteriaceae* in all batches were in the range of 10^{-2} to 10^{-3} (Fig 3C), which rapidly decreased after fermentation in all batches and they were not recovered after one week of ripening. The rapid decline in pH value and probably bacteriocin produced by *Lactobacillus casei* (Fadda and Vignolo, 2002) and antagonistic characteristics of *S. xylosus* (Villani *et al*., 1997) could suppress these pathogenic *Enterobacteriaceae*.Similar results have been reported by González and Díez (2002) in Spanish dry cured sausage.

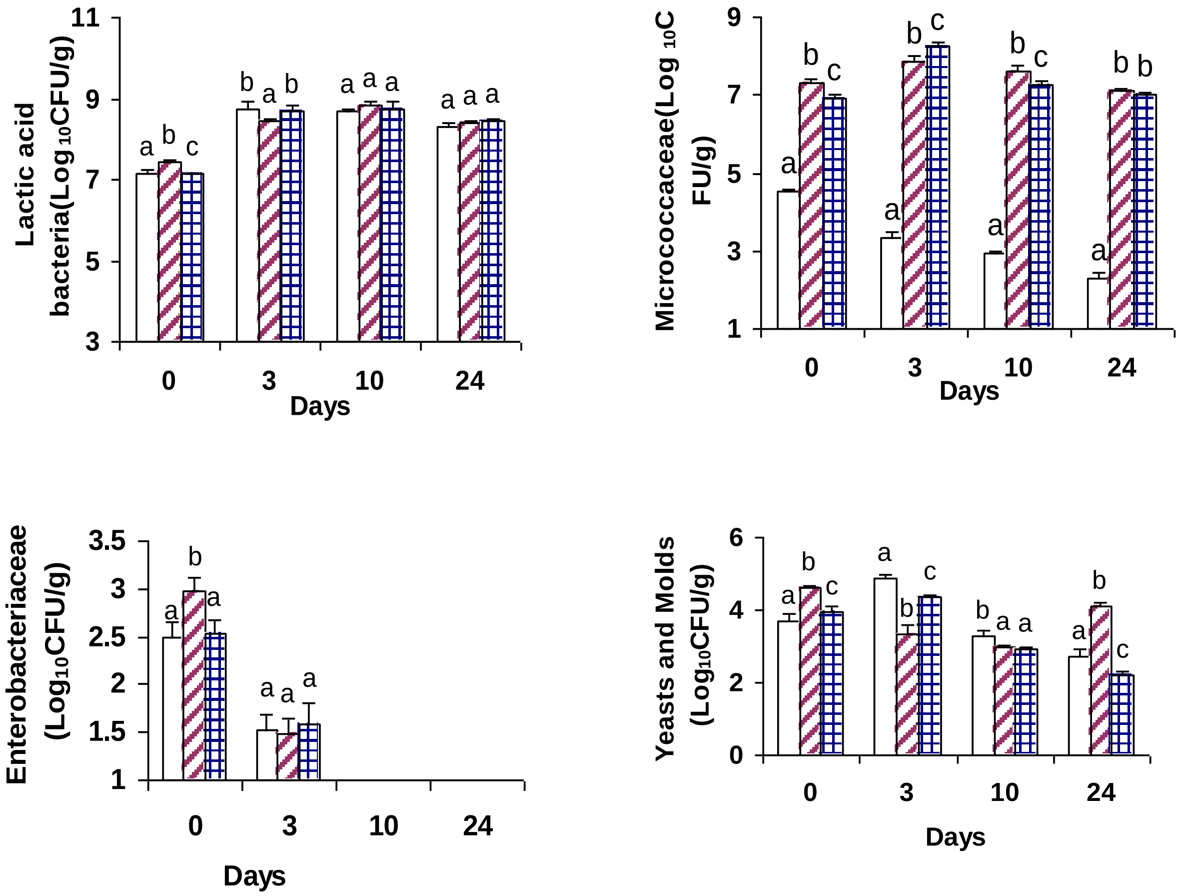


Fig 3- Effects of mixed cultures on microbial changes during fermentation and ripening of batches: □ CHS-LC; ▨ CHS-SX; ▦ CHS-SL. Means with different lower case letters within treatments at the same processing period (day) are significantly different (P<0.05).

The initial counts for yeast and molds were in the range of 10^{-3} to10^{-4} (Fig 3D), however they couldn't exceed 10^{4} in any batch during fermentation and ripening of dry fermented Chinese-style sausages. Batch CHS-SL showed the lowest (P<0.05) counts, mean while batch CHS-SX showed the highest (P<0.05). In parallel, Yeast and molds are usually reported in dry fermented sausages and salami products due to long ripening period under low temperature and controlled RH condition (Baldini *et al*., 2000; Coppola *et al*., 2000; Moretti *et al*., 2004).

Results for sensory evaluation of dry fermented Chinese-style sausages are shown in Fig 4.

No significant differences (P>0.05) were found for saltiness and hardness in all batches. The quality characteristics such as appearance, red color, flavor and overall acceptability were significantly higher (p<0.05) for mixed culture batch (CHS-SL).By contrast, treatment CHS-LC was found most sour and least sweet (P<0.05)

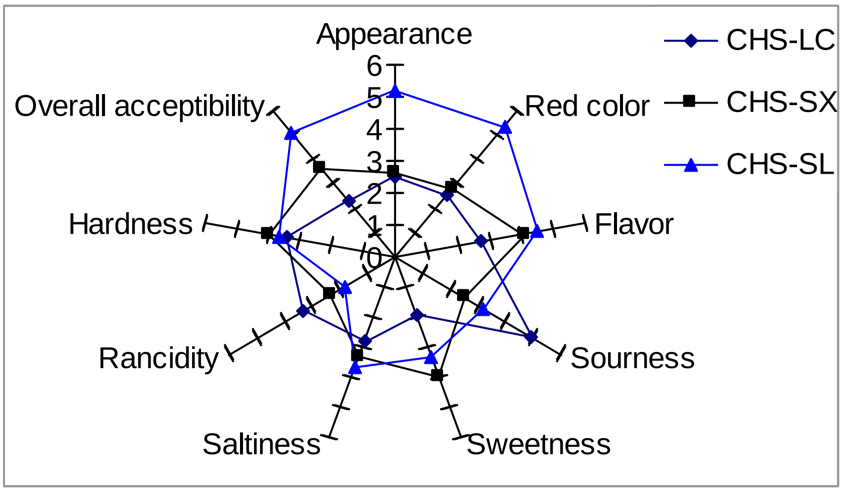


Fig 4- Effects of mixed starter cultures on the sensory characteristics of dry fermented Chinese-style sausages.

as well as poor in overall acceptability. The lower scoring (P<0.05) for overall acceptability of treatment CHS-LC clearly reflected that the panels couldn't accept the sour taste due to lower (P<0.05) pH value (Table 2). Since *Staphylococcus xylosus* could produce volatile compounds in dry fermented sausage (Montle *et al*. 1996, Søndergaard and Stahnke 2002, Olesen *et al.*, 2004) and *L. casei* could have proteolytic activity (Fadda *et al*. 2001, 2002) thus the combination of these two starter culture seems to have good effects on flavor of dry fermented sausage.

CONCLUSIONS

The proximate composition of final products has been found similar to each other and none of the culture combination influenced greatly on the proximate composition of final products. The mixed culture of *L. casei.* subspp.*casei*-1.001 and *S. xylosus* -12 as starter for dry fermented Chinese-style sausage produced higher amount of proteolytic-related parameters such as α-amino acid (AAN) and non protein nitrogen (NPN), which could influence the flavor of final products. Likewise, mixed starter culture produced higher (P<0.05) red color and optimum pH, which are considered to be more desirable characteristics for such kind of products. The growth of *Micrococcaceae* during sausage processing was not influenced by mixed starter culture, while they were inhibited by *L. casei.* subspp.*casei*-1.001 used as single starter culture. In contrast, mixed starter culture showed better inhibitory effects on the growth of pathogenic *Enterobacteriaceae* as well as yeast and molds in final products ensuring the microbial safety in product.

The sensory analysis result showed that the product inoculated with mixed starter culture was superior (P<0.05) in appearance, red color, flavor, rancidity and overall acceptability. The degree of sourness influenced on overall acceptability of these kinds of products. Hence the use of mixed starter culture in dry fermented Chinese-style sausage successively controlled the pH at optimum level, which was more acceptable than highly acidic products. Likewise the hardness of mixed starter culture batch was slightly lower but statistically not significant (P>0.05). This work clearly demonstrated that the mixed starter culture of *L. casei.* subspp.*casei*-1.001 and *S.xylosus*-12 would be more appropriate than a single starter to improve the quality characteristics of dry fermented Chinese-style sausage.

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