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EFFECTS OF SODIUM LACTATE ON THE QUALITY OF DRY FERMENTED CHINESE-STYLE SAUSAGES INOCULATED WITH LAB CULTURE OF *PEDIOCOCCUS PENTOSACEUS*-ATCC 33316

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ABSTRACT

The efficacy of sodium lactate (SL) at 0-3% (w/w) concentration supplemented in four batches of dry fermented Chinese-style sausages inoculated with lactic acid bacteria (LAB) culture of *Pediococcus pentosaceus*-ATCC 33316 were analyzed for physico-chemicals and microbiological quality of products. The proximate composition like moisture, sugar and salt content in samples containing ≥2% level of SL were significantly increased ($p<0.05$), mean while the protein and fat contents were decreased ($p<0.05$). Likewise, the weight losses, water activity (a_w) and pH declination in sausages were significantly reduced ($p<0.05$) in final products containing ≥2% SL as compare to the control. Besides, a highly positive correlation ($r = 0.986$, $p<0.05$) was observed between residual nitrite content and concentration of SL in sausages. The SL concentration at ≥2% in samples showed significantly lower ($p<0.05$) values for non protein nitrogen (NPN), thiobarbituric acid (TBA) value and total volatile basic nitrogen (TVBN) during ripening of sausages. In contrast, SL at any concentration had no significant ($p>0.05$) effect on Hunter *L, a* & *b* values of samples but significantly ($p<0.05$) improved the hardness of sausage. Moreover, significant inhibitory ($p<0.05$) effects on microorganisms like total plate counts (TPC), lactic acid bacteria (LAB), yeast and moulds, *Micrococcaceae* and *Enterobacteriaceae* counts has been shown at concentration 2% or above. Result of the sensory analysis showed that the sample treated with SL at 2% was superior in quality parameters. The findings suggested that an optimum level of SL at 2% (w/w) concentration could be supplemented in sausage batter as a pH regulator, antioxidant and antimicrobial agent to produce a high quality of dry fermented Chinese-style sausage inoculated with LAB culture of *P. pentosaceus*-ATCC 33316 in commercial scale.

Key words: Sodium Lactate; Dry fermented Chinese-style sausage; *P. pentosaceus*-ATCC 33316; pH; Antimicrobial; Lipid oxidation

INTRODUCTION

Sodium lactate is generally recognized as safe (GRAS) food additive by the US Code of Federal Regulations, 21 CFR. SL produced by microbial fermentation is the sodium salt of natural lactic acid (L^+) and is a normal component of muscle tissue. It has been used to control the growth of certain microorganisms during storage, pH control, enhance flavor and processing yields of meat products (Doores, 1993; Stekelenburg and Kant-Muermans, 2001). Hence the use of SL as a functional food additive in varieties of foods including meat and meat products have already been reported (Stekelenburg and Kant-Muermans, 2001; Lin and Lin, 2002). The growth of lactic acid bacteria (LAB) and *L. monocytogenes* is dependant mainly on water activity of the product, storage temperature and residual nitrite to some extent in final products. In numerous studies it has been noted that SL as a food additive to control the water activity in food products (Brewer *et al*. 1991; Stekelenburg and Kant-Muermans, 2001). Likewise, addition of SL at 1-3 % showed lower number of total plate counts in low fat Chinese-style sausages (Lin and Lin, 2002). Besides, its antibacterial action against plate counts, *Leuconostoc* spp., LAB (Wang, 2000), *E. coli* 0157:H7 (Byrne *et al*. 2002) and *Listeria monocytogenes* and *Salmonella* spp. (Mbandi and Shelef 2002) have been reported. SL could also be used as a pH regulator in meat particularly in fermented meat products (Deumier and Collignan 2003). Maca, *et al*. (1999) have been suggested that SL at 3% concentration could be the optimum level to act as a bacteriostatic agent, antioxidant and color stabilizer in processing and storage of cooked beef system.

The dry fermented sausage inoculated with LAB strains have been considered as one of the safer meat products. However its sour taste due to acidic pH and lipid oxidation caused by long time ripening could be causative factors of dislike by

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several consumers. Therefore in this study SL was supplemented in sausage batters to control the pH declination as well as lipid oxidation in dry fermented Chinese-style sausage inoculated with *P. pentosaceus*-ATCC 33316 as meat starter. Furthermore, other objective of this work was to optimize the concentration of SL on the basis of physicochemicals as well as microbiological quality parameters of products.

MATERIALS AND METHODS

Preparation of inoculums

P. pentosaceus-ATCC 33316 was sub-cultured twice in (de Man Rogosa Sharpe agar)MRS broth for 3 days at 30^{o}C then harvested by centrifugation (Model HSC-20RA, Tumen, China) at 10,000g for 15 min at 4°C, subsequently washed twice with 20mM phosphate buffer (pH 7.0) and finally resuspended in the same buffer (10% of initial volume) (Fadda *et al*. 2002). The number of bacterial cells in each suspension was adjusted to reach at the range of 10^7 CFU/g in sausage batter by using spectrophotometer (Model WFZ-UV-2100, Unico^{TM}). The optical density was measured at 680nm then adjusted at 2.0 (Fadda *et al*. 1998). The average corresponding viable cells for *P. pentosaceus*-ATCC 33316 at optical density 2.0 was 4.4 x10^9 CFU/ml.

Manufacturing of sausages

Fresh pork ham parts and back fat was purchased from the local supermarket in Wuxi. All the required chemicals and a food grade sodium lactate (50-60%, pH 7.5-8.5) were procured from chemical store of SYU, China.

Four batches (CHS-PP) of dry fermented Chinese-style sausages inoculating *P. pentosaceus*-ATCC 33316 were manufactured in laboratory scale. For this purpose a fresh boneless pork ham part and back fat were frozen over night (below -10^{o}C) to mince through a meat mincer (coarse size, through a 9.5mm plate) then the batters were mixed with all curing ingredients including equal quantity of starter culture to all batches and SL concentration levels at 0, 1, 2 and 3% (w/w) respectively. The sample supplemented no SL (0%) served as the control. The curing ingredients per 100g of sausage batter consisted the following proportion: pork lean (80%), pork back fat (20%), sugar 8.0g, salt 2.0g, sodium nitrite 0.012g, sodium tripolyphosphate 0.2g, chilled water 10g, five spice powder 0.3g, MSG powder 0.5g, sodium erythorbate 0.05g, potassium sorbate 0.2g , rice wine 1.0g and white pepper powder 0.1g. All the sausage batters (each of 10 kg in pilot plant scale) were cured for 24 hr at 4-5^{o}C then stuffed into collagen casing, which were linked and tied each ends with the help of sterilized cotton thread. Each and every link of sausages were hung up inside the pilot plant scale convenient fermentation chamber for fermentation (22 ±1^{o}C and 90-95% RH for 3 days), first stage ripening/ drying (16±1^{o}C and 80-85% RH for one week) and final ripening/drying (12 ±1^{o}C and 70-75% RH for two weeks) .Finally the sausage samples were vacuum packaged using a vacuum packaging machine (MULTIVAC, Model A300/16, Germany) and kept in frozen condition (below -10^{o}C) until analysis were done.

Sampling and statistical analysis

A complete sampling plan was assigned during processing as follow; initially just before the stuffing into casing (0 day), after fermentation stage (3rd day), after one week of ripening (10th day), and final product (24th day). Five links of sausages from each step were taken then packed in vacuum packaging and kept in deep freeze below -10^{o}C until analyses were completed. One way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) was applied to compare the mean data of triplicates. Pearson's correlation coefficient, when ever needed was calculated to show the significant correlation between the treatments. All statistical analyses were performed using the SPSS 11.5.0 (2002).

Proximate analysis

Moisture content, Crude Protein, Crude Fat, Ash content were measured by the methods described in AOAC (1997) and salt content and total sugar was measured by the method of Rangana (1991) and James (1995) respectively.

Chemical analysis

The nitrite was determined by following the method described in AOAC (1997), pH and total volatile basic nitrogen (TVBN) was measured by the method of Wang (2000) and Pearson (1968) respectively. Similarly, thiobarbituric acid (TBA) value was measured by the method of Beuge and Aust (1978) ; Vasavada *et al*. (2003) and non protein nitrogen (NPN) was measured by the method of Dierick *et al*. (1974).

Physical analysis

Physical analyses were done to determine the following parameters. Weight losses (Bloukas, *et al*. 1997b); Water activity (Lin, and Lin, 2002); Hunter color values (*L*, *a*, *b*) were measured by color difference meter (Model TC–PII G, Beijing Optical

Instruments, China); Texture Profile Analysis (TPA) by using a texture analyzer (Model TAXT2i, Stable Micro System, England) (Bourne 1978; Lin and Chao 2001).

Microbiological analysis

Microbiological analyses were carried out according to the procedure of APHA (1992).

Sensory analysis

Sensory evaluation was carried out in a descriptive way using a 6-point hedonic scale for each attribute ranging from 0 for lowest to 6 for highest preference (Moretti *et al*. 2004).

RESULTS AND DISCUSSION

Effects on proximate composition

A proximate composition for SL supplemented at 0 -3% (w/w) in dry fermented Chinese-style sausages is presented in Table 1. The samples treated with SL at concentration 2% or above showed significantly ($p<0.05$) higher moisture values, while protein and fat content were found significantly ($p<0.05$) lower. The moisturizing property of SL has clearly been shown in final products.

Effects on weight losses and water activity (a_w)

The weight losses in samples treated with 0-3% SL and subsequently inoculated with starter *P. pentosaceus*-ATCC 33316 is shown in Fig 1, where weight losses was observed in the range of 46.96 to 50.78%.at 24 days of processing time. Use of SL at above 2% significantly reduced ($p<0.05$) the weight losses in final products. This could be due to humectants property of SL (Bloukas *et al*. 1997a).

The highest water activity ($a_w = 0.84$) was observed in control sample, while the lowest ($a_w = 0.82$) in sample treated with 3% SL (Fig 1). Therefore, significantly lower ($p<0.05$) a_w values were noted in SL treated samples. Similarly, Lin and Lin (2002) also reported that the Chinese style sausage containing 3% SL had a significantly lower ($p<0.05$) a_w value than any other treatments during storage for 12 weeks.

Effects on pH

The effect of SL on pH of dry fermented Chinese–style sausages inoculated with *P. pentosaceus* ATCC 33316 is given in Fig 2.

Initially, the pH in all sausage batters was almost identical but the changes have been occurred during fermentation and ripening process. The pH value trend to be always decreasing in order and significantly ($p<0.05$) lower value was found in control as compared to SL treated samples. The result was in line with the finding of Deumier and Collignan (2003), who applied 1.8% of SL in dry fermented Chicken sausages to control the pH of final products.

Effects on nitrite and NPN value

The effects of SL on residual nitrite and NPN values in dry fermented sausages are shown in Fig 3. Highly positive correlation ($r = 0.986$, $p<0.05$) were observed between residual nitrite content and concentration of SL used in samples. The samples containing over 2% SL had higher amount of residual nitrite as compare with the control, though the highest concentration of nitrite was below 7 mg/kg. Samelis *et al*. (1998) have been reported be-

Table 1: Proximate composition of sausage inoculated with P. pentosaceus-ATCC 33316 and treated with different concentration of sodium lactate (Mean ± Standard deviation)

Parameters^{x,y}	Sodium lactate concentration (%)			
	SL 0	SL 1	SL 2	SL 3
Moisture	$27.07\pm.6^{a}$	28.66 ± 0.6^{ab}	29.23 ± 0.9^{bc}	30.93 ± 1.7^{c}
Protein	41.56 ± 0.6^{b}	41.04 ± 1.4^{b}	40.37 ± 1.0^{b}	38.13 ± 1.4^{a}
Fat	38.97 ± 0.5^{c}	38.4 ± 0.2^{bc}	37.70 ± 0.4^{a}	37.80 ± 0.2^{ab}
Ash	6.9 ± 0.04^{a}	6.77 ± 0.2^{a}	6.8 ± 0.05^{a}	6.94 ± 0.04^{b}
Salt	3.58 ± 0.2^{a}	3.75 ± 0.1^{ab}	3.95 ± 0.1^{b}	4.06 ± 0.2^{b}
Total Sugar	11.78 ± 0.3^{a}	11.98 ± 0.4^{a}	12.31 ± 0.4^{ab}	12.75 ± 0.3^{b}

[x] The means with different superscripts in the same row are significantly different ($p<0.05$).
[y] All the proximate compositions are expressed in g/100 g of dry weight basis.

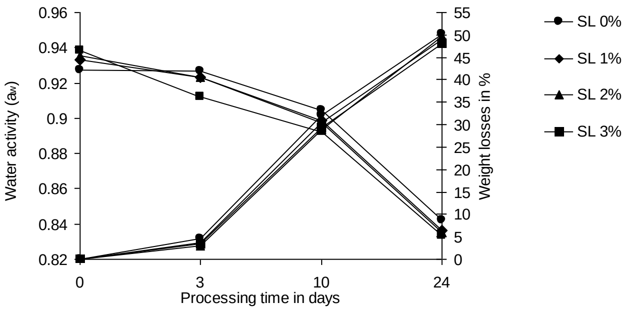

Figure 1: Effect of sodium lactate on weight losses and changes in water activity (a_w) of dry fermented Chinese-style sausage inoculated with *P. pentosaceus*-ATCC 33316

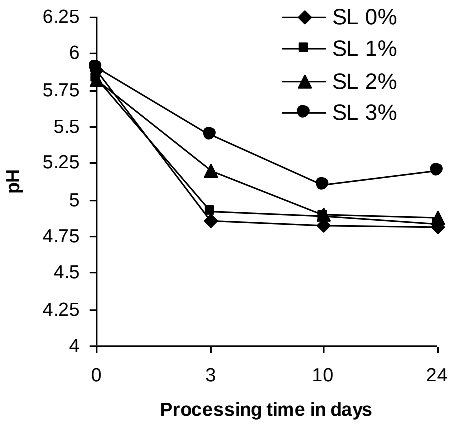

Figure 2: Effect of sodium lactate on pH during ripening of dry fermented Chinese-style sausages inoculated with *P. pentosaceus* ATCC 33316

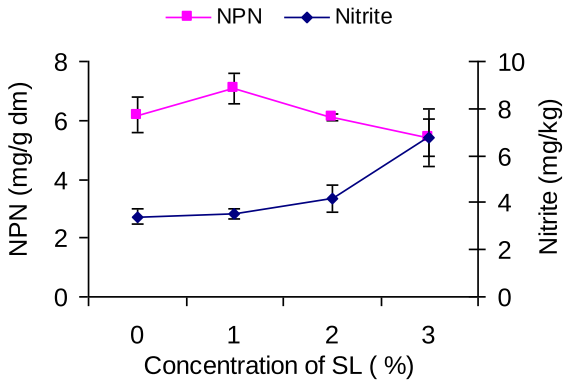

Figure 3: Effect of sodium lactate on residual nitrite content and NPN value in dry fermented Chinese style sausages inoculated with *P. pentosaceus* ATCC 33316

low 10ppm nitrite in Greek salami ripened for 28 days.

In other hand, the sample added with 3% SL showed the significantly ($p<0.05$) lowest NPN value (5.4mg/100g), whilst below 2% of SL had no effect. It revealed that the antimicrobial action of SL against *P. pentosaceus* ATCC 33316 and other spoilages bacteria that could control the muscle proteolysis and hence lower the NPN values in SL supplemented products.

Effects on Lipid oxidation and TVBN

The effects of SL on fat oxidation and TVBN values during ripening of dry fermented Chinese-style sausages are shown in Fig 4. TBA values in samples treated above 2% SL were significantly lower ($p<0.05$) (1.02 to 0.96mgMDA/kg) as compared to the value obtained for the control (1.24mgMDA/kg) at 24 days after ripening. In contrast to our finding, Maca *et al*. (1999) found no changes in TBA values during storage of vacuum packaged beef that added with 3 or 4% of SL. However, 2% of SL in ground pork had significant inhibitory effect on fat oxidation during refrigeration storage (Tan and Shelef 2002).

On the other hand, the trends of TVBN were increasing over time of processing, though the samples containing 2% or above SL had significantly lowered ($p<0.05$) the TVBN values in final products (19.44 - 22.46mg/100g). Since, TVBN is related with the microbial activity in muscle foods and apparently showed that the antimicrobial action of SL, which could influence the microbiological ecology in dry fermented sausages that ultimately, influenced the TVBN values. Wang (2000) also found significantly lower value for TVBN in Chinese-style sausage stored at 20^0C.

Effects on Hunter colour values

The effects of SL concentrations on Hunter *L*, *a*, and *b* colour values of dry fermented Chinese-style sausages are shown in Fig 5. All types of colour values were seen significantly decreasing in order ($p<0.05$) over ripening. The samples treated with SL at any concentration had no effects ($p>0.05$) on red colour of sausages. Other authors have also been reported that the addition of SL (1-3%) had no effects on Hunter *L*, *a*, *b* values (Brewer *et al*. 1991).

Effects on texture profile

The effect of SL on textural properties of dry fermented Chinese-style sausages inoculated with *P. pentosaceus* ATCC 33316 is presented in Table 2. Hardness is considered as the most important parameter in dry fermented sausage. In this study, a significant inverse correlation ($p<0.05$) was established between hardness and concentration of SL that added in samples. It revealed that the different concentration of SL could effect on the texture of dry sausages. The concentration of SL over 2% in products resulted a significant ($p<0.05$) level of tenderness in products. Besides, other parameters such as adhesiveness, springiness and resilience were not affected but slightly low values for cohesiveness, gumminess and chewiness were noticed in SL added samples. The finding was in line with the result of McGee *et al*.(2003), who has reported tendered product as compare to control for precooked beef injected with 2% of SL. In contrast, Bloukas *et al*. (1997a) found no difference for hard-

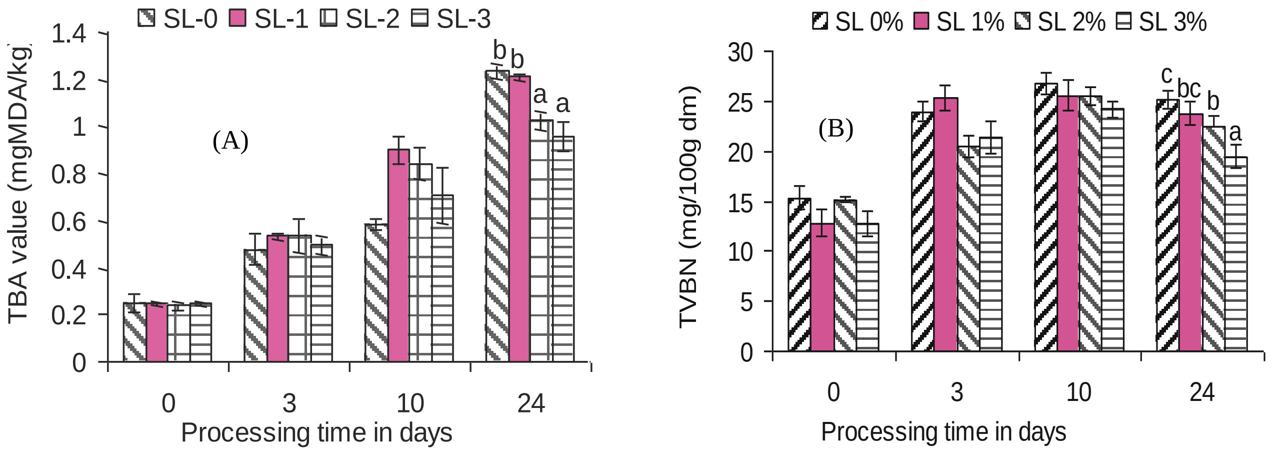

Figure 4: Effects of sodium lactate on lipid oxidation (Fig 4A) and changes in TVBN value (Fig 4B) during processing of dry fermented Chinese-style sausages inoculated with P. pentosaceus ATCC 33316. Bar having different lower case letters within same processing period are significantly different (p<0.05)

ness in low fat frankfurters supplemented with SL.

Microbiological analysis

The effects of SL on the growth of different microorganisms during ripening of dry fermented Chinese-style sausages inoculated with *P. pentosaceus* ATCC 33316 is shown in Fig 6, where Fig 6A represents the changes in TPC, *Enterobacteriaceae* and yeast and molds during processing of sausages. Initially, the TPC in the sausage batters was found in the range 10^7 CFU/g, which significantly increased to a level 10^8CFU/g just after fermentation then decreased significantly ($p<0.05$) during ripening of products that added with SL.

Particularly, SL above 2% showed a significant ($p<0.05$) inhibitory effects on TPC. Likewise, numbers of pathogenic *Enterobacteriaceae* was significantly decreased by 2 log unit after fermentation, which thereafter did not appear in any samples. Besides, the yeast and molds counts were also found to be decreased ($p<0.05$) in SL treated samples as compared to control. The inhibitory effects of SL on different meat system have been reported by several authors (Papadopoulos *et al*. 1991; Maca *et al*. 1999).

The initial counts of LAB and *Micrococcaceae* in sausage batters were in the range of 10^7 and 10^5 CFU/g respectively. After fermentation the numbers were increased up to 10^8 CFU/g for LAB and almost unchanged for *Micrococcaceae* except in control. The LAB counts and *Micrococcaceae* were significantly decreased ($p<0.05$) in all SL treated products as shown in Fig 6B. A highly negative correlation ($r = 0.789$, $p<0.01$) between LAB

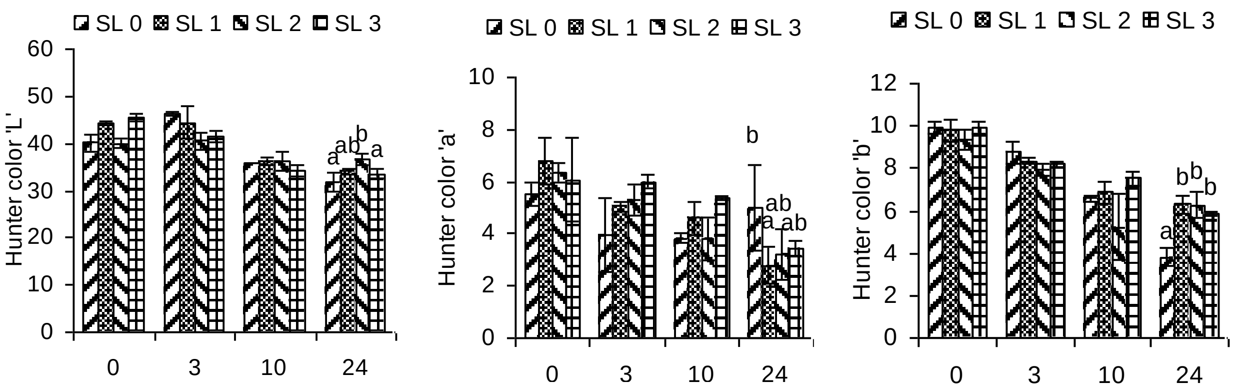

Figure 5: Effects of SL on the Hunter color value (L, a, & b) during processing of dry fermented Chinese-style sausages inoculated with *P. pentosaceus* ATCC 33316. Bar having different lower case letters within the same processing period are significantly different (p<0.05).

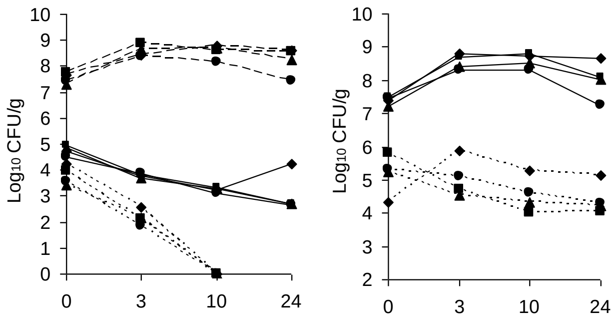

Figure 6: Effects of sodium lactate at concentration (SL 0% —¨—, SL 1% —■—, SL 2% —▲—, SL 3% —●—) on Total plate counts (— —), Yeast & Molds (—), and Enterobacteriaceae (---) in Fig (A) and on LAB (—) and Micrococcaceae (----) in Fig (B) in ripening of dry fermented Chinese-style sausage inoculated with P. pentosaceous ATCC 33316.

Table 3: Mean sensory scores of 15 panelists for dry fermented Chinese-style sausages treated with different SL concentration

Parameters[x]	SL-0%	SL-1%	SL-2%	SL-3%
Appearance	3.2[a]	3.5[a]	3.8[a]	3.8[a]
Red color	3.6[a]	3.9[a]	3.9[a]	3.5[a]
Flavor	3.7[a]	3.5[a]	3.7[a]	3.7[a]
Sourness	4.3[b]	3.1[a]	3.0[a]	2.9[a]
Sweetness	2.6[a]	3.0[ab]	3.4[b]	4.2[c]
Saltiness	3.1[a]	3.5[a]	3.1[a]	3.2[a]
Rancidity	2.6[b]	1.9[a]	1.9[a]	1.7[a]
Hardness	4.6[c]	3.9[b]	3.1[a]	3.1[a]
After taste	3.9[b]	3.5[ab]	4.0[b]	3.0[a]
Overall quality	3.7[ab]	3.3[a]	4.3[b]	4.3[b]

[x] Means with different superscripts in the same row are significantly different ($p<0.05$).

counts and pH in products during processing clearly showed the inverse relation between them and thus the SL could be used as pH controlling agent by inhibiting the growth of LAB in fermented meat products. Such inhibitory effect of SL against LAB has been elucidated in different meat system (Deumier and Collignan 2003). From this study, above 2% of SL supplementation in sausages showed strong antimicrobial effects against bacteria as well as yeast and molds in dry fermented Chinese-style sausages inoculated with *P. pentosaceous* ATCC 33316.

Effects on sensory quality

The mean sensory scores of dry fermented Chinese -style sausages supplemented with sodium lactate at concentrations 0-3% (v/w) is given in Table 3.

The sensory attributes like appearance, red colour, flavor and saltiness were insignificant ($p>0.05$) in final products, while the rancidity, hardness and sourness values were significantly decreased ($p<0.05$) in samples supplemented with sodium lactate. It is likely seems that the sourness and texture are major quality parameters to influence the overall quality of sausage. Hence, the sensory result revealed that the product treated with 2% SL showed a better effect on sensory quality. The samples treated with 3% SL gained less mean scores, which is probably due to an objectionable taste produced by SL in product. An objectionable taste was reported in low fat frankfurter treated with higher amount of SL but 2% of SL treated frankfurter showed best sensory score (Bloukas *et al*. 1997b). The functional properties such as antioxidant, color protection, texture improvement, pH regulator in different food systems supplemented with SL at 2-4% have already been reported (Brewer *et al*. 1991; Maca *et al*. 1999; Deumier and Collignan 2003).

Table 2: Effects of sodium lactate on the textural properties of dry fermented Chinese-style sausage inoculated with *P. pentosaceus* ATCC 33316

TPA[x]	Sodium lactate concentration (%)			
	SL 0	**SL 1**	**SL 2**	**SL 3**
Hardness (g)	8665.6[b]±773.6	8458.04[ab]±812.2	9037.61[b]±389.8	7364.23[a]±304.1
Adhesiveness(g)	-1334.5[a]±392.7	-1750.4[a]±676.6	-1666.2[a]±633.6	-1923.07[a]±704.4
Springiness(mm)	0.87[a]±0.134	0.79[a]±0.07	0.788[a]±0.04	0.833[a]±0.07
Cohesiveness	0.48[b]±0.04	0.387[a]±0.03	0.462[b]±0.04	0.426[ab]±0.02
Gumminess(g)	4154.7[b]±265.7	3270.6[a]±370.6	4183.01[b]±487.3	3143.39[a]±181.6
Chewiness (g x mm)	3849.2[b]±820.0	2675.9[a]±232.1	3303.2[ab]± 504.9	2610.11[a]±66.1
Resilience	0.076[a]±0.003	0.072[a]±0.003	0.084[a]±0.008	0.079[a]±0.023

[x] Texture profile analysis, where the means with the different superscripts are significantly different ($p<0.05$)

CONCLUSIONS

Supplementation of sodium lactate (SL) at 2 % concentration or more showed an effective actions against the lipid oxidation, microbial growth and pH declination in dry fermented Chinese-style sausages that inoculated with LAB strain of *P. pentosaceous* ATCC 33316. Moreover, SL at 2% (v/w) in dry sausage improved the textural properties, sensory attributes to some extend and microbial safety level in final product. However, higher concentration of SL (i.e. above 2%) is not recommended, as it produced an objectionable taste in final product that could affect on the sensory quality of sausage. Therefore, SL at 2% (v/w) could be recommended for manufacturing of better and hygienic quality of dry fermented Chinese-style sausage.

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