INFLUENCE OF SUCROSE LEVELS AND INOCULATION OF *LACTOBACILLUS PLANTARUM* ON QUALITY OF ISAN SAUSAGE (THAI FERMENTED PORK SAUSAGE)

F. J. Tan, N. Hongthong, W. Chumngoen, D. C. Liu, and M. T. Chen *

Department of Animal Science, National Chung Hsing University, Taichung, 402, Taiwan

Abstract – This study was to evaluate the influence of sucrose levels (0.3% and 1.2%) and inoculation of *Lactobacillus plantarum* on the qualities of Isan sausage. The results showed that the sausages that inoculated with starter cultures (0.3SLp and 1.2SLp) fastened the fermentation process than the ones without inoculation (0.3S and 1.2S) in accordance to the lower pH in 4 days, whereas there was no significant difference in pH values between the samples with different sucrose adding levels (0.3SLp vs. 1.2SLp and 0.3S vs. 1.2S). Inoculated samples had significantly lower thiobarbituric acid reactive substances (TBARS) and volatile basic nitrogen (VBN) values than the ones without inoculation. The growth pattern of total plate count (TPC) was in consistency with the growth of lactic acid bacteria (LAB). For the sensory evaluation, the panels preferred the sensory characteristics of the inoculated and 1.2% sucrose-added sausages.

Key Words – Isan sausage, *Lactobacillus plantarum*, sucrose.

I. INTRODUCTION

Isan sausage, also known as “Sai Krok Prew”, is a typical fermented sausage abundantly produced and commonly consumed in Thailand. The fermentation of certain traditional fermented sausages in Thailand and other areas is frequently achieved by natural, wild-type lactic acid bacteria such as *lactobacilli* and *pediococci* that originate from the raw meat [1]. However, the production of fermented meat products with naturally occurring lactic acid bacteria often results in products with inconsistent qualities and even unsafe products and it might also include some spoilage species or sometimes even pathogenic microorganisms [2]. The microorganisms used for meat fermentation commonly consist of a group of lactic acid bacteria (LAB), such as *Lactobacillus plantarum*, *L. sakei*, *L. curvatus*, and *L. pentosus* as starter cultures. The changes during fermentation include decrease in pH, reduction of pathogenic bacteria, organic acid production, solubilization and increase of hydrolysis of myofibrillar and sarcoplasmic proteins, color, and flavor development [3]. Carbohydrates are used as the obligatory carbon and energy source in microbial fermentation for meat products, resulting in lactic acid accumulation that reduces pH. Simple sugars, such as glucose and sucrose, are favored by fermentable bacteria because these carbohydrates can be readily transported through the bacterial cell wall [4]. Therefore, the aim of this study was to investigate the influence of sucrose levels (0.3% and 1.2%) and inoculation of *L. plantarum* on physicochemical, microbiological and sensory characteristic of Isan sausage.

II. MATERIALS AND METHODS

L. plantarum ATCC 14917 was subcultured in deMan Rogosa Sharpe (MRS) broth and purified by successively streaking on MRS agar plates. Isan sausage was prepared according to the methods of Phromraksa *et al.* [5]. Sucrose (0.3% or 1.2%) for treatments of 3S and 12S, and cell suspension of starter culture (10 mL/kg) for treatments of 3SLp and 12SLp respectively, then incubated in an incubator (30°C and 75% RH) until day 4 for fermentation purpose, then stored at 4°C until day 28. Physicochemical analysis included pH value, thiobarbituric acid reactive substances (TBARS) and volatile basic nitrogen (VBN). Microbiological analysis were determined accordingly Wanangkarn *et al.* [2] for enumeration of total plate counts (TPC) and lactic bacteria counts (LAB). Sensory evaluation, at the day 4, 14, and 28, sausages were cooked until the internal temperature reached and held at 75°C for 8 min and then served to a sensory panel and evaluated using a 1–7 point scale test, with 1 and 7 representing extremely dislike and extremely like, respectively, for the attributes.

III. RESULTS AND DISCUSSION

In this study, sausages were sampled at day 0 (after stuffing), day 2 (fermentation process), day 4 (end-fermentation), and day 7, 14, 21, and 28 (storage period).

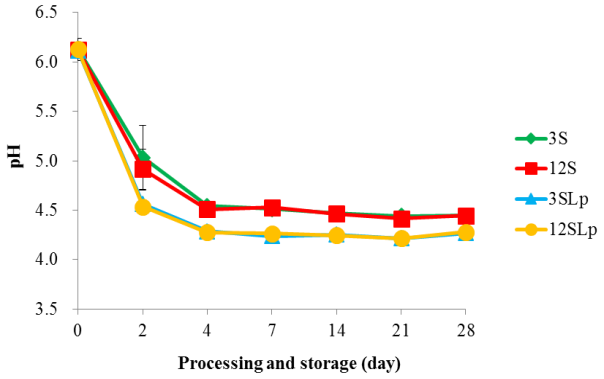

Figure 1. Changes in pH value of Isan sausages with/without starter culture and sucrose during processing and storage.
3S=0.3% of sucrose and no starter culture; 12S=1.2% of sucrose and no starter culture; 3SLp=0.3% of sucrose and starter culture (*L. plantarum*); and 12SLp=1.2% of sucrose and starter culture (*L. plantarum*).

Fig. 1 showed a rapid decrease in pH values especially between day 0 and day 4. At day 0, after stuffing the pH value of sausage was 6.12–6.13 without significant difference ($P>0.05$) between treatments. Sausage without inoculation (0.3S and 1.2S) decreased to 4.51–4.54 on day 4, and then remained stable until the end of storage on day 28 ($P>0.05$). The pH values of the inoculated sausages followed a similar pattern but were significantly lower than the non-inoculated ones. The reduction in pH was probably due to the growth of lactic acid bacteria in samples. The pH value of the Iranian fermented sausage which inoculated with *L. plantarum* and *L. fermentum* was quickly reduced during the 4-day fermentation probably due to the fermentation to organic acids of carbohydrates occurred in sausages by these lactic acid bacteria [6].

The TBARS values of all samples increased ($P<0.05$) throughout storage time as expected (Fig. 2a). The slightly increased in TBARS value occurred in sausages without inoculation (0.3S and 1.2S) from 1.31–1.38 mg MDA/kg at day 0 up to 2.36–2.60 mg MDA/kg at day 28. The inoculated samples had TBARS values increased from 1.09–1.12 mg MDA/kg at day 0 to 1.56–2.12 mg MDA/kg at day 28. Sawitzki *et al.* [7] reported that Milano-type salami inoculated with *L. plantarum* strain AJ2 had lower TBARS values when compared to the control throughout the storage time and suggest that *L. plantarum*, produced a good effect on the oxidative stability of lipids. Sausages with 1.2% sucrose addition (1.2S and 1.2SLp) showed lower TBARS values lower than sausage with 0.3% sucrose addition.

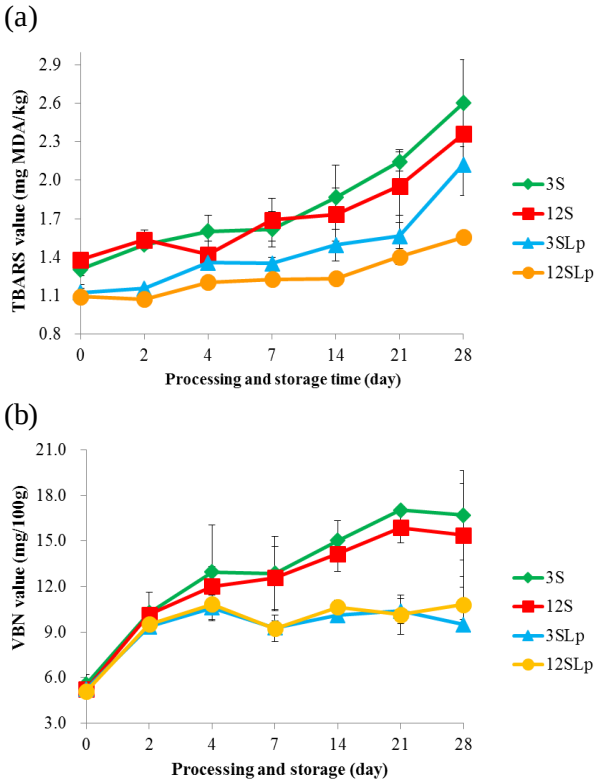

Figure 2. Changes in (a) TBARS and (b) VBN values of Isan sausages with/without starter culture and sucrose during processing and storage.

The VBN values of the non-inoculated samples increased rapidly from 5.25–5.59 mg/100 g to 12.02–12.95 mg/100 g after 4-day fermentation (Fig. 2b), while those of samples with LAB increased slowly from 5.11–5.26 to around 10 mg/100 g. The VBN of all treatments increased significantly ($P<0.05$) during 4-day fermentation. The VBN of inoculated sausages remained stable during storage whereas the VBN of the non-

inoculated ones increased significantly (P<0.05) during storage period. This study agreed with other studies [8].

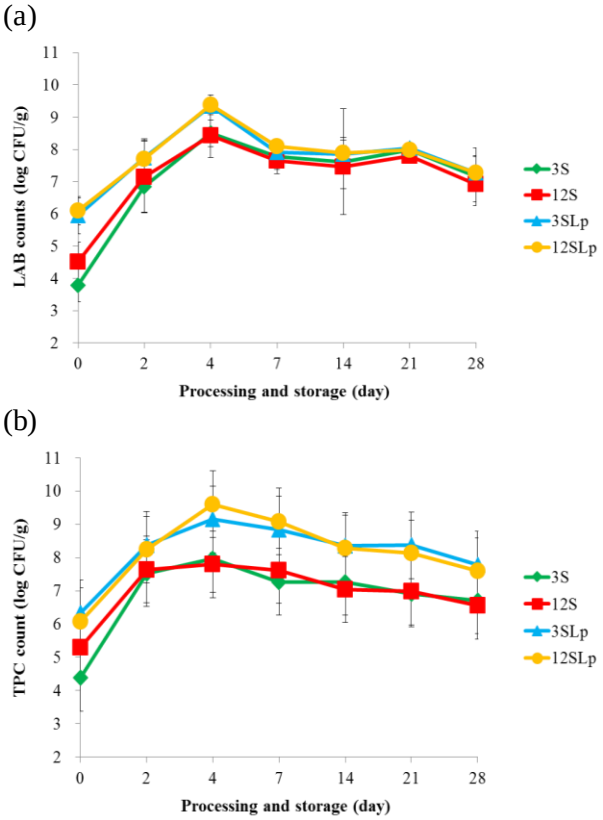

Figure 3. Changes in counts of (a) LAB and (b) TPC of Isan sausages with/without starter culture and sucrose during processing and storage.

Fig. 3 showed changes in counts of (a) LAB and (b) TPC. During fermentation, the LAB counts (Fig. 3a) increased significantly from 3.79–6.09 log CFU/g at day 0 to 8.44–9.39 log CFU /g at day 4 (P<0.05), and then decreased to 6.92–7.28 log CFU /g at the end of storage. Samples without inoculation had lower LAB counts than the inoculated ones (3.79–4.52 log CFU/g vs. 5.94–6.09 log CFU/g) and the counts of the inoculated samples increased to 8.50–8.44 log CFU/g and 9.34–9.39 log CFU/g, respectively, day 4. During storage, at day 7 all of treatments decreased from day 4 around 1 log CFU/g unit and remained stable to day 21, and then decreased significantly until the end of storage and there was no significant difference (P>0.05) between the samples with different sucrose adding levels. The growth pattern of total plate count was in accordance with the expected growth of lactic acid bacteria (LAB) in all of processes [9].

The TPC (Fig. 3b) of all treatments increased significantly with the increased fermentation time (P<0.05). During storage, the TPC counts of all treatments significantly decreased (P<0.05). In general, sausages with addition of starter culture had higher microbial counts than the non-inoculated ones.

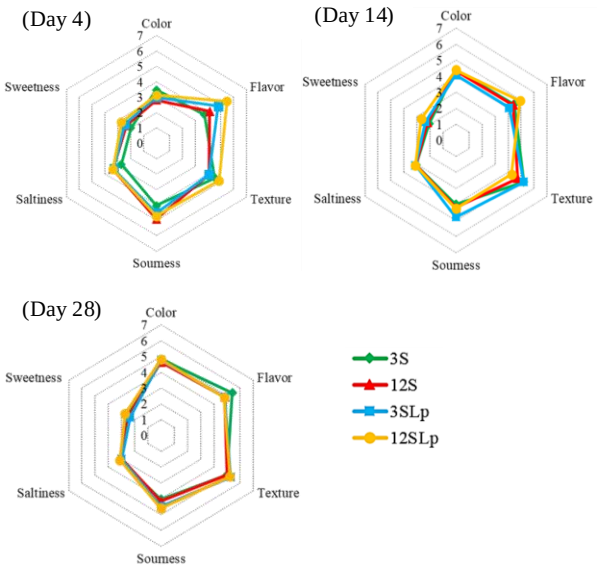

Figure 4. Sensory evaluation of Isan sausages with/without starter culture and sucrose at day 4, day 14, and day 28 using the intensity method.

In this study, two sensory evaluation methods including intensity evaluation method and like-and-dislike method were conducted and illustrated in Fig. 4 and Fig. 5, respectively. Using the intensity evaluation method (Fig. 4), it was found the colors of sausages became darker, especially after fermentation (P<0.05) and flavors of the non-inoculated samples increased after storage for 14 days. At day 4 after fermentation, the inoculated samples had higher flavor intensity scores (P<0.05) than the non-inoculated ones (4.80–5.47 vs. 3.73–4.13, respectively) as evaluated using the intensity method. Inoculated ones were preferred by the panel and also give high score of acceptability as compared to the non-inoculated ones (Fig. 5). The intensity method, score of flavor were kept stable with increasing storage process. The flavor score decreased with increasing storage process from day 4 (4.27–5.53) decreased to day 28 (3.07–4.47). This result agreed with TBARS values, at day 28,

the TBARS increased to the high values in all treatments.

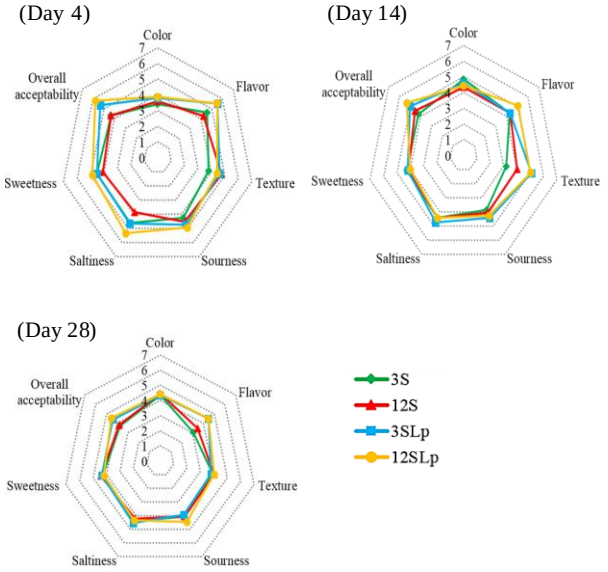

Figure 5. Sensory evaluation of Isan sausages with/without starter culture and sucrose at day 4, day 14, and day 28 using the like-dislike method.

Off-flavors of sausages increased when storage time increased. After fermentation, the texture scores increased ($P<0.05$) from 4.07–4.87 (a little bit hard) at day 4 up to 5.00–5.27 (hard) at day 28 (Fig. 4), and this results agreed with Barbut [10], who reported that the lower pH in cooked salami-type sausages resulted in more disruption of protein coagulates formed during heating (hardness). Other sensory characteristics including sourness, saltiness, and sweetness did not significantly ($P>0.05$) change using intensity and like-and-dislike evaluation methods.

I. CONCLUSION

In conclusion, a combination of adding sucrose at level of 1.2% and inoculation of *L. plantarum* at level of 7 log CFU/g not only enhances the physicochemical, microbiological qualities of the Isan sausages, but also increases the sensory acceptance of products.

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