EFFECTS OF STARTER CULTURES ON MICROBIAL CHANGES DURING PRODUCTION OF NOVEL THAI FERMENTED BEEF SNACK STICKS

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Abstract – We investigated the effects of bacteriocin-producing lactic acid bacteria (LAB) starter cultures on microbial changes during production of novel Thai style beef snack sticks fermented at 30°C for 24 h, followed by 2-h heating at 60°C, and 6-d drying at 14°C. Treatments included Control (no starter culture), Commercial (with Pacovis RCI-47 starter culture, Germany), P536 (with *Pediococcus pentosaceus* TISTR 536), and P2 (with *Lactococcus lactis* subsp. *lactis* P 2). At each replication, three beef sticks from each treatment were randomly taken for LAB, yeasts and molds, and *Escherichea coli*, coliforms, *Staphylococcus aureus*, and *Salmonella* evaluation, initially before fermentation, after 24-h fermentation before heating, after 2-h heating, and after 2, 4, and 6-d drying, respectively. Data were analyzed as a Randomized Complete Block Design with three replications. Starter cultures help to reduce coliforms and yeasts. The heating process at 60°C for 2 h after fermentation resulted in the reduction of LAB (0.5-1.5 log cfu/g), yeasts (0.1-0.6 log cfu/g), and coliforms (0.1-1.2 log cfu/g). By the end of production, however, the number of LAB was 6.7-7.7 log cfu/g. Treatment added with *P. pentosaceus* TISTR 536 tended to provide more LAB than that added with *L. lactis spp. lactis* P2.

Key Words – Bacteriocins, Heat-treated, Probiotic

I. INTRODUCTION

Mum, a Northeastern Thai fermented meat product, is traditionally produced from ground beef, water buffalo meat, or pork and their liver or spleen mixed together with fresh ground garlic, cooked rice, and salt. Batter is stuffed into large diameter natural casings such as bladders or small intestines and tied before being naturally fermented and air dried at ambient temperature for several days or until tangy. Mum is usually consumed raw, but sometimes grilled. Beef snack sticks are very popular among the Western countries with the production approximately 500 million sticks each year [1]. The application of hurdle technology for fermented sausage production, for example, by using starter cultures [2, 3, 4] or by applying heat treatment after fermentation [5, 6, 7] can improve product quality and safety. Thermotolerant bioprotective LAB cultures have been isolated from cooked sausages [8]. *P. pentosaceus* TISTR 536, a probiotic potential LAB isolated from typical Thai fermented pork sausages called Nham, was reported to be able to grow and produce pediocin PA-1 after heat treatment at 50°C [9,10]. Isolated from fish intestines, *L. lactis* subsp. *lactis* P 2 was also found to potentially be probiotic, but producing Nisin Z [11]. We investigated effects of two bacteriocin-producing cultures compared to commercial starter culture and to that naturally fermented on microbial changes during production (fermentation, heating, and drying) of newly developed Thai style beef with liver (Mum) snack sticks.

II. MATERIALS AND METHODS

Four treatments of beef snack sticks, consisting of 81% ground beef plates, 8.1% beef liver, 8.1% fresh ground garlic, 2.0% cooked rice, 0.13% nitrited salt, 0.07% sodium phosphate, 0.3% monosodium glutamate, and 0.2% glucose were produced without starter culture, with 6 log cfu/g of Pacovis RCI-47 starter culture (Lot No. 8.850.04, Pacovis, GmbH, Germany), 6 log cfu/g of *P. pentosaceus* TISTR 536, or 6 log cfu/g of *L. lactis* subsp. Lactis P 2. Each sausage treatment was prepared in a bowl chopper (Seydelmann, Germany), stuffed (TALSA stuffer #0542 MODEL H26PA 380/50/3, EU) into 19-mm diameter collagen casing (Nippi, Japan), tied into 6-inch length, and transferred to Type KA 50 air-conditioned maturing cabinet (Südtronic M. Schaaf + Co., Germany). Fermentation was done at 30°C and 90%

relative humidity (RH) for 24 h, then heat treated at 60°C and 70%RH for 2 h. Temperature was then reduced to 12-14°C with 65% RH for another 6-d drying. Three beef sticks from each treatment were randomly taken for LAB, yeasts and molds, *E. coli*, coliforms, *S. aureus*, and *Salmonella* evaluation [12] on D0 (initially before fermentation), D1BH (after 24-h fermentation before heating), D1AH (after 2-h heating), D3, D5, and D7 (after 2, 4, and 6-d drying, respectively). Data were analyzed as a Randomized Complete Block Design with three replications using SPSS version 13 [13].

III. RESULTS AND DISCUSSION

Initially before fermentation, LAB for all treatments were similar (6.1-6.8 log cfu/g, p>.05, Table 1. As expected, after 24-h fermentation, LAB of all treatments increased (p<.05). Commercial had more LAB than P536, Control, and P2, respectively (p<.05). LAB in commercially available starter culture might be able to grow better than those in P536 and P2 which are laboratory prepared. Interestingly, P2 had the lowest LAB (p<.05). This might be due to the origin of LAB in P2 which were isolated from fish intestines [11]. Therefore, it could not grow well in beef raw materials compared to *P. pentosaceus* TISTR 536, which was isolated from fermented meat product or to those naturally presented in control. Previous studies [14, 15] reported that *Pediococcus* are the most prevalent LAB found in traditionally produced Mum, this Northeastern Thai fermented meat product. In addition, it could also be due to effect of Nisin Z produced by P2 against some naturally presented LAB, resulting in lower LAB in this treatment.

At D1AH, the 2-h heat treatment at 60°C resulted in decreasing of LAB from 0.5 to 1.5 log cfu/g, especially in Commercial (from 9 log cfu/g to 7.4 log cfu/g, p<.05). Ensoy et al. [7] reported a 1.5 log cfu/g reduction of LAB in Turkish fermented sausages, Sucuk, fermented and heat treated at 70°C until internal temperature reached 55°C for 5 min. Among treatments, however, we found no difference in LAB counts (p>.05). During ripening and drying at 14°C and 65% RH for 6 days, no difference in LAB was found at each sampling period (p>.05). No difference in LAB among treatments was also observed during D3 and D5 of production. However, P2 tended to have lower LAB, especially by the end of production (p<.05). The lower number of LAB in P2 after fermentation might result in continued slower growth and less LAB. The reason for more LAB in Control than in P2 by D7 indicated a better growth of the naturally occurring LAB, which could be *Pediococcus* species [14,15]. In addition, heating for 2 h could injure LAB resulted in slower growth, especially in P2 which had lower LAB number since the fermentation process. However, by the end of production, LAB of all treatments reached 6.7-7.7 log cfu/g. For health benefits, probiotic products must contain a high number of probiotic cultures at approximately 6 log cfu/g [16].

Table 1. Changes in Lactic acid bacteria during 7 days of Mum snack stick production

Day (D)	Lactic acid bacteria (log cfu/g)			
	Control	Commercial	P536	P2
D0	6.1 b	6.8 b	6.6 c	6.5 b
D1BH	8.2 ax	9.0 aw	8.4 ax	7.9 ay
D1AH	7.7 ab	7.4 b	7.7 ab	7.4 ab
D3	7.3 ab	7.8 ab	6.9 bc	6.4 b
D5	7.1 ab	7.3 b	6.8 bc	6.5 b
D7	7.6 abw	7.7 abw	7.4 abcw	6.7 abx

Control = sticks without starter culture added
Commercial = sticks added with 6 log cfu/g of commercial starter culture (Pacovis RCI-47, Germany)
P536 = sticks added with 6 log cfu/g of *Pediococcus pentosaceus* TISTR 536
P2 = sticks added with 6 log cfu/g of *Lactococcus lactis spp. lactis* P 2
BH = after 24- h fermentation at 30°C and before heating
AH = after heating at 60°C for 2 h
abc means in a column without common letters differ (p<.05)
wxy means in a row without common letters differ (p<.05)

The initial counts of yeasts in all treatments were similar (3.6-4.1 log cfu/g, p>.05, Table 2). These levels are similar to those (4.9 log cfu/g) found in Mum traditionally produced from native Thai beef [17]. In Western style fermented product, Ensoy et al. [7] reported initial yeast counts of 5.7, 5.5, and 9.9 log cfu/g in Sucuk produced with starter cultures and without, respectively. After fermentation, we found an increase (p<.05) in yeasts of all treatments, especially in Control which had

higher (p<.05) yeast counts than those in P536. Applying heat to Mum snack sticks after fermentation tended to reduce numbers of yeasts (0.1-0.6 log cfu/g, p>.05, Table 2). The decrease in yeasts of about 1 log cfu/g was found in Sucuk produced with heat treatment [7]. During drying (D3-7), yeasts tended to increase (p>.05). By D7, yeast counts in all treatments were 5.4-6.3 log cfu/g, p>.05). The growth of yeasts and molds can be found in dry or semi-dry fermented meat products [18]. Yeasts and molds of fermented sausages were reported at 2-3 log cfu/cm^2 initially, but after 25-d ripening, yeasts increased to 5-6 log cfu/cm^2, while molds were 6-7 log cfu/cm^2 [19]. Mold counts during processing in our study, however, were est. <1 log cfu/g (data not shown), which was similarly observed in traditionally produced Mum [17].

Table 2. Changes in Yeasts during 7 days of Mum snack stick production

Day	Yeasts (log cfu/g)			
	Control	Commercial	P536	P2
D0	4.1 c	4.1 b	3.6 b	3.9 b
D1BH	6.1 abw	5.7 abwx	5.2 abx	5.5 abwx
D1AH	6.0 ab	5.1 ab	5.0 ab	4.9 ab
D3	5.9 bw	4.6 abx	5.0 abwx	5.0 abwx
D5	6.8 a	5.8 a	6.1 a	6.1 a
D7	6.5 ab	5.9 a	5.4 a	5.7 a

Control = sticks without starter culture added
Commercial = sticks added with 6 log cfu/g of commercial starter culture (Pacovis RCI-47, Germany)
P536 = sticks added with 6 log cfu/g of *Pediococcus pentosaceus* TISTR 536
P2 = sticks added with 6 log cfu/g of *Lactococcus lactis spp. lactis* P 2
BH = after 24- h fermentation at 30°C and before heating
AH = after heating at 60°C for 2 h
abc means in a column without common letters differ (p<.05)
wx means in a row without common letters differ (p<.05)

E. coli counts in all treatments were est. <1 log cfu/g (data not shown). In traditionally produced Mum made from native Thai beef, the *E. coli* counts of93 MPN/g were reported [17]. For coliforms, initially in all treatments, there were 3.8-4.4 log cfu/g (p>.05, Table 3). After fermentation, coliforms tended to increase, especially in Control (p<.05). But no difference (p>.05) in coliforms was found among Commercial, P536, and P2. While coliform counts in Control were similar to those found in P536 and P2, they were higher (p<.05) than those in Commercial. Heating for 2 h after fermentation seemed to decrease (p>.05) coliforms in all treatments (0.1-1.2 log cfu/g). In general, coliforms in starter culture added treatments decreased continuously after heating until D7. In contrast, coliforms in Control seemed to decrease (p>.05) after heating, but then increased (p>.05) during drying. On D5, Commercial had the lowest coliform counts (p<.05). By D7, coliform counts in Control (5.3 log cfu/g) were higher (p<.05) than those (3.2 log cfu/g) in Commercial, but were not different (p>.05) from P536 and P2 (3.8 and 3.8 log cfu/g, respectively). Furthermore, no difference (p>.05) in coliforms was found in all starter culture added treatments. Finally, for all treatments, at each stage of production, *S. aureus* counts were est. <1.0 log cfu/g, while negative results were obtained for *Salmonella* detection (data are not shown).

Table 3. Changes in coliform counts during 7 days of Mum snack stick production

Day	Coliforms (log cfu/g)			
	Control	Commercial	P536	P2
D0	4.4 b	3.8	3.9	4.1
D1BH	6.1 aw	4.2 x	5.2 wx	5.0 wx
D1AH	4.9 ab	4.1	4.1	4.7
D3	5.1 ab	3.8	4.7	4.6
D5	5.0 abw	3.3 x	4.1 w	4.1 w
D7	5.3 abw	3.2 x	3.8 wx	3.8 wx

Control = sticks without starter culture added
Commercial = sticks added with 6 log cfu/g of commercial starter culture (Pacovis RCI-47, Germany)
P536 = sticks added with 6 log cfu/g of *Pediococcus pentosaceus* TISTR 536
P2 = sticks added with 6 log cfu/g of *Lactococcus lactis spp. lactis* P 2
BH = after 24- h fermentation at 30°C and before heating
AH = after heating at 60°C for 2 h
ab means in a column without common letters differ (p<.05)
wx means in a row without common letters differ (p<.05)

IV. CONCLUSION

Utilization of starter cultures helps to reduce coliforms and yeasts in snack sticks produced by adopting Western fermented sausage production technology. The heating process at 60°C for 2 h after fermentation resulting in 0.5-1.5 log cfu/g reduction of LAB, 0.1-0.6 log cfu/g of yeasts, and 0.1-1.2 log cfu/g of coliforms. However, the number of LAB by the end of processing were high (6.7-7.7 log cfu/g). For treatments added

with bacteriocin-producing probiotic potential LAB, treatment added with *P. pentosaceus* TISTR 536 tends to provide more LAB than that added with *L. lactis spp. lactis* P2. The presence of *P. pentosaceus* TISTR 536 and probiotic properties in the final product, as well as its effect on pathogenic control during production need to be further investigated.

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