EFFECT OF GARLIC ON LACTIC ACID AND BACTERIOCINS PRODUCTION OF BACTERIOCIN-PRODUCING LACTIC ACID BACTERIA ASSOCIATED IN NHAM (THAI FERMENTED MEAT) DURING FERMENTATION

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Introduction

Garlic, a flavouring food plant, is used as ingredient in various meat products, especially in Nham. The amount of garlic used in Nham is about 3-5%. Many reports are published on the effects of garlic on the growth of lactic acid bacteria (LAB) starter cultures and their lactic acid production in various kinds of fermented meat products including Nham (Swetwiwathana *et al.*, 1999a). Moreover, many publications implied some inhibitory effects of garlic on various food poisoning bacteria (Dababneh and Al-Delaimy, 1984; Swetwiwathana *et al.*, 1999b). Thus, we realized that garlic must also present some effects on lactic acid and even bacteriocins production of bacteriocin-producing LAB associated in Nham such as pediocin PA-1 producer (*Pediococcus pentosaceus* TISTR 536) and nisin Z producers (*Lactococcus lactis* subsp. *lactis* N100 and N190) (Swetwiwathana *et al.*, 2003). Hence, this paper is to report the effect of garlic on lactic acid and bacteriocins production from Nham-associated pediocin PA-1 and nisin Z producers during Nham fermentation.

Materials and Methods

Microorganisms: A strain of *P. pentosaceus* TISTR 536 (Bangkok, MIRCENS) and 2 strains of *Lc. lactis* subsp. *lactis* N100 and N190, which were isolated from Nham and were confirmed to produce pediocin PA-1 and nisin Z respectively (Swetwiwathana *et al.*, 2003), were used for this study. *Listeria innocua* LTH 3096 was used as the indicator for bacteriocin activity determination.

Medium: MRS broth was used as a cultivation medium for *P. pentosaceus* TISTR 536, *Lc. lactis* subsp. *lactis* N100 and N190. The incubation condition for all cultures was 30°C for 24 h. Trypticase soy broth + 0.6 % yeast extract (TSBYE) was used as a cultivation medium for *L. innocua* LTH 3096. The incubation condition for all cultures was 30°C for 24 h. Trypticase soy agar + 0.6 % yeast extract (TSAYE) was used as an agar base and Lactobacilli agar AOAC (LAA, Difco) was used for bacteriocin activity determination.

Study on the production of lactic acid and bacteriocin by *P. pentosaceus* TISTR 536, *L. lactis* subsp. *lactis* N100 and N190 during Nham fermentation:

Preparation of Nham was carried out in 1 kg batches per each strain of isolated bacteriocin-producing LAB from Nham as starter culture. The samples without starter culture (control batch) were also prepared for natural fermentation. The recipe of Nham was described in Swetwiwathana *et al.*, (1999 a, b)

The mixed ingredients of Nham samples (with and without garlic), except the control sample, were inoculated with either strain of *P. pentosaceus* TISTR 536, *L. lactis* subsp. *lactis* N100 or N190 at a level of 10^6 cfu/g and mixed with a KitchenAid model K45SS for 1 min. Approximately 25 g of both Nham control batch mixture without starter culture and Nham with starter cultures were stuffed into 10x14 cm plastic bags. Then, the mixture was squeezed to the closed bag end and tightly wrapped with a rubber band. All samples were fermented at 30°C for 3 days. Two bags per sample were examined every 12 h of fermentation for pH (Swetwiwathana *et al.*, 1999a), lactic acid production (Lotong and Swetwiwathana, 1990) and bacteriocin production.

Determination of the concentration of antimicrobial product during Nham fermentation:

Two bags per each studied condition of Nham samples were sampling for 10 g. The ratio of 1:1 dilution of Nham sample homogenates (Nham 10 g: distilled water 10 ml) were prepared. After centrifugation of each Nham sample homogenate (2,700 x g for 10 min), the supernatant was adjusted to pH 6 with 5 N NaOH and then filter-sterilized (0.20 µm pore-size polysulfone). Bacteriocins activity of each cell-free filtrate was determined by using spot-on-lawn technique as described by Mayr-Harting *et al.* (Mayr-Harting *et al.*, 1972).

Results and Discussion

Among the use of the three LAB strains (*P. pentosaceus* TISTR536, *L lactis* subsp. *lactis* N100 and N190) for Nham fermentation, pediocin PA-1 producer (*P. pentosaceus* TISTR536) was found to be the best strain as a starter culture of Nham production. The strain exhibited good results in decreasing pH, higher lactic acid production (Figure 1) and more stability of its pediocin PA-1 produced (Table 1), while nisin Z producer of both N100 and N190 showed less decrease in pH and lactic acid production including unstable bacteriocin production during Nham fermentation. This might be

Table 1: Proximate composition and colour values of Chinese meatballs with various amounts of mechanically deboned bullfrog meat (MDBM) added.

Items	Mechanically deboned bullfrog meat added (%)				
	0	7.5	15.0	22.5	30.0
Moisture	59.95±0.75^{w}	60.61±0.74^{wx}	61.03±0.80^{xy}	61.43±0.69^{xy}	62.43±1.24^{y}
Protein	24.07±0.46^{w}	23.76±0.35^{wx}	23.39±0.69^{x}	22.56±0.36^{y}	21.87±0.20^{z}
Fat	17.22±0.88^{w}	15.44±0.29^{x}	15.04±0.26^{x}	14.23±0.14^{y}	14.16±0.04^{y}
Ash	2.40±0.45	2.03±0.97	1.99±0.46	1.93±0.46	1.92±0.27
L* value	63.66±1.17^{w}	62.46±2.08^{wx}	62.23±1.28^{wx}	60.82±0.76^{x}	59.14±1.29^{y}
a* value	2.82±0.78	2.35±0.14	2.51±0.11	2.50±0.04	2.62±0.05
b* value	11.54±0.07	11.62±0.46	11.64±0.31	11.71±0.48	11.79±0.40

$^{w, x, y, z}$ Means within a row that have different superscripts are significantly different ($p < 0.05$).

Table 2: Changes in water holding capacity and cooking loss of Chinese meatballs with various amounts mechanically deboned bullfrog meat (MDBM) added during refrigerated storage.

MDBM added (%)	Water holding capacity	Cooking loss
0	37.30±4.99^{a}	4.27±0.71^{a}
7.5	30.21±2.60^{b}	4.33±0.96^{a}
15.0	27.71±5.69^{bc}	4.70±1.20^{a}
22.5	24.29±3.99^{cd}	6.14±2.35^{ab}
30.0	21.91±3.65^{d}	7.11±2.11^{b}

$^{a, b, c, d}$ Means within a column that have different superscripts are significantly different ($p < 0.05$).

Table 3: Changes in pH and TBA values of Chinese meatball with various amounts of mechanically deboned bullfrog meat (MDBM) added during refrigerated storage.

MDBM added (%)	Storage time (day)			Storage time (day)		
	0	7	14	0	7	14
	pH values			TBA (mg malonaldehyde/kg sample)		
0	6.37±0.06^{az}	6.11±0.04^{ay}	5.81±0.05^{ax}	1.59±0.64^{dx}	1.70±0.14^{dx}	1.89±0.18^{dx}
7.5	6.43±0.02^{bz}	6.17±0.00^{by}	5.87±0.02^{bx}	3.85±0.65^{cy}	3.93±0.14^{cy}	4.17±0.11^{cxy}
15.0	6.47±0.0^{bcz}	6.22±0.01^{cy}	5.99±0.01^{cx}	4.09±0.73^{cy}	4.19±0.12^{cy}	4.38±0.06^{cxy}
22.5	6.51±0.01^{cdz}	6.29±0.02^{dy}	6.02±0.01^{cx}	5.20±0.87^{bx}	5.40±0.37^{bx}	5.60±0.46^{bx}
30.0	6.54±0.01^{dz}	6.37±0.02^{ey}	6.08±0.01^{dx}	6.50±1.22^{ay}	6.58±0.80^{ay}	6.77±1.01^{axy}

$^{a, b, c, d}$ Means within a column in the same test that have different superscripts are significantly different ($p < 0.05$).
$^{x, y, z}$ Means within a row in the same test that have different superscripts are significantly different ($p < 0.05$).

Conclusions

In conclusion, Chinese meatballs that had 7.5% of mechanically deboned *Rana catesbeiana* meat added dur manufacturing was acceptable based on the physic-chemical qualities.

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