INDICATOR BACTERIA AND EFFICACY OF CRUDE BACTERIOCINS FROM *LACTOCOCCUS LACTIS* TISTR 1401 ON CHICKEN MEATBALLS SHELF LIFE

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Abstract- The objectives of this study were to obtain dominant bacterial strains from in-house prepared chicken meatballs to be used as indicator bacteria, and to use 10-fold concentrated crude (CCB) and freeze dried crude bacteriocins (FDCB) produced from *Lactococcus lactis* TISTR 1401 to extend shelf life of chicken meatballs by mixing in the meatball batter. In comparison with commercial nisin, 0.2% each form of bacteriocins was mixed in chicken meatball batter prior to forming in hot water. The meatballs were aerobically and vacuum packed and stored at 4 ^oC for 21 days, samples were taken every 3 days for pH measurement and microbial enumeration. The inhibition activities of CCB, FDCB and nisin against the most dominant strain of *Bacillus* spp. (85%) were 3200, 6400 and 6400 AU/ml, respectively. According to the Thai FDA microbial standard count of $\leq$ 5.0 log cfu/g food, it was found that with bacteriocins adding, the meatballs could be kept at least 3 days longer than control meatballs when packed in both conditions; 18 days for aerobically packed and 21 days for vacuum packed meatballs. The FDCB and nisin added meatballs had slightly lower ($p < 0.05$) microbial counts and pH values than CCB meatballs. However, it could be mentioned that an inhibition efficacy of both forms of crude bacteriocins was comparable to that of commercial nisin.

Index Terms- chicken meatball batter, crude bacteriocins, indicator bacteria, shelf life extension.

I. INTRODUCTION

Bacteriocins are ribosomal peptides produced by Gram-positive bacteria and can kill or inhibit growth of many closely related bacteria. Among the Gram-positive bacteria, lactic acid bacteria (LAB) is one of the most essential bacteria used as a reservoir for antimicrobial peptide production in particular bacteriocins for food applications. Bacteriocins produced from LAB are used as food additives. For instance, nisin is widely used in foods and has generally been regarded as safe (GRAS) bacteriocins which affectively inhibit the growth of both spoilage and pathogenic bacteria (de Arauz, Jozala, Mazzola & Penna, 2009; Ross, Morgan & Hill, 2002). Generally, nisin is not widely used in meat products, because of binding of nisin to meat particles in meat systems (Deegan, Cotter, Hill & Ross, 2006), less activity of bacteriocins in meat products is expected due to binding of bacteriocin peptide with meat proteins during heat process. However, Intarapichet and Gosaarak (2008) revealed the success of using crude bacteriocins produced from *Lactococcus lactic* TISTR 1401 to extend the shelf life of meatballs by coating on the surface of cooked pork meatballs. Therefore, application of crude bacteriocins in the meatball batter prior to meatball forming in hot water should be an alternative means of application to extend shelf life of cooked meat products.

Finely comminuted low fat meatballs are one of the major economically important meat products with a high consumption in Thailand. For further investigation of the efficacy of crude bacteriocins produced from *Lc. lactic* TISTR 1401 on the extension of the meatball shelf life, concentrated and dried forms of crude bacteriocins were mixed in the chicken meatball batter prior to forming in hot water. In addition, isolated and identified dominant bacteria from chicken meatballs were used as indicator strains for testing an inhibition ability of these crude bacteriocins. Therefore, the objectives of this study were to test an efficacy of crude bacteriocins produced from *Lc. lactic* TISTR 1401 against dominant bacterial strains identified from in-house chicken meatballs and to prolong shelf life of the meatballs by mixing crude bacteriocins in the meatball batter in the forms of concentrated liquid and freeze dried powder prior to meatball forming.

II. MATERIALS AND METHODS

Obtaining indicator bacteria from chicken meatballs. Chicken meatballs were made from the meat batter containing 10 kg chicken meat, 2% salt, 0.4% sodium phosphate, 15% ice and 5% tapioca starch. The batter was formed into meatballs in 60 ^oC water for 5 min then in 80 ^oC water for 20 min, dried at room temperature for 10 min, and aerobically packed in plastic bag. Bacterial collection and isolation were obtained from 24 h meatballs and identification was done according to bacterial morphology and biochemical reactions with API system. Three dominant bacterial strains were used to test against crude bacteriocins.

Preparation of Bacteriocins and inhibition test against indicator bacteria. Bacteriocins were produced by the selected LAB strain of *Lactococcus lactis* TISTR 1401 obtained from Thailand Institute of Scientific and Technological Research (TISTR) in MRS broth supplemented with 2% (w/v) yeast extract, 2% glucose and 0.2% meat extract, incubated at 37 °C and controlled pH at 6.5. Fermentation broth was centrifuged at 12,000xg at 4 °C for 20 min. The supernatant was neutralized to pH 6.5 with 1 M NaOH, and catalase of 1 mg/ml of final concentration was added and incubated for 30 min at room temperature, then heated at 80 °C for 20 min, cooled and stored at 4 °C until use. Crude bacteriocins were tested against dominant bacterial strains by agar well diffusion methods.

Crude bacteriocin supernatant was evaporated at 40 °C to obtain 10-fold concentrated crude bacteriocins (CCB), and freeze dried to obtain freeze dried crude bacteriocins (FDCB). Into three separate batches of chicken meatball batter, 0.2% of CCB, FDCB and commercial nisin were mixed prior to meatball forming. The treated and control meatballs were aerobically and vacuum packed in plastic bags and kept at 4 °C. The meatball samples were taken for analyses every 3 days for 21 days.

Microbiological analysis and pH measurement. The serial dilutions were plated on PCA agar for total aerobic plate count and incubated at 37 °C for 12-48 h, MRS agar for LAB and incubated at 30 °C for 12-48 h, pHs of the samples were measured using probe type pH meter.

Statistical analysis. Analysis of variance ($p = 0.05$) and DMRT were performed using SAS for windows. Two replications of the experiment were performed with triplicate analyses per replication.

III. RESULTS AND DISCUSSON

Indicator bacteria from chicken meatballs. Three most dominant bacteria strains were identified as 3% of *Staphylococcus lentus*, 12% of *Stap. saprophyticus* and 85% of *Bacillus spp.* (PN-3). Inhibition activity of CCB, FDCB and nisin against *Bacillus spp.* indicator bacteria were 3,200, 6,400 and 6,400 AU/ml, respectively.

Antimicrobial property of crude bacteriocins mixed in chicken meatball batter. Total plate counts (TPC) of the aerobically and vacuum packed chicken meatballs, kept at 4 °C are shown in Fig. 1. It was clearly shown that TPC of the meatballs mixed with both forms of concentrated crude bacteriocins were similar to those mixed with nisin and lower than those of control meatballs throughout the storage period. The FDCB form obviously gave better microbial retardation than CCB form.

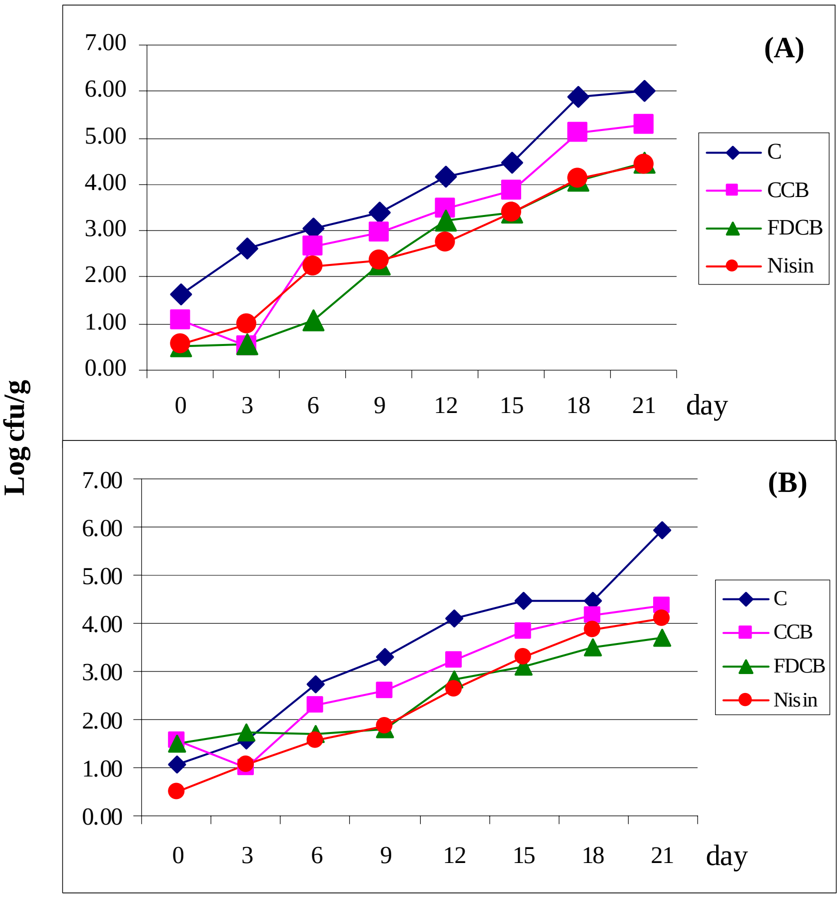

Fig. 1. Total microbial contents of chicken meatballs mixed with bacteriocins, stored at 4 °C. A = aerobic packaging, B = vacuum packaging, C = control, CCB = concentrated crude bacteriocin, FDCB = freeze dried crude bacteriocins.

According to the Thai FDA standard for microbial count of ≤ 5.0 log cfu/g food sample, the treated and aerobically packed meatballs could be kept at least 18 days with microbial counts of 5.14, 4.09 and 4.12 log cfu/g for CCB, FDCB and nisin, respectively, while the control sample had the count of 4.46 log cfu/g in 15 days (Fig. 1A). Longer storage times were found for the vacuum packed meatballs that the treated meatballs could be kept longer than 21 days with TPC of 4.36, 3.71 and 4.09 log cfu/g for CCB, FDCB and nisin treated, respectively, while the control sample had TPC of 4.47 log cfu/g in 18 days (Fig. 1B). The inhibition efficacy of these concentrated and dried powder forms of bacteriocins used in cooked food, processed in hot water at 80 °C was in agreement with the results reported by Morgan, Galvin, Kelly and Hill (1999).

The LAB counts of all meatballs mixed with bacteriocins were detected between days 6-9 in both packaging conditions and gradually increased thereafter with about 2 log cycles lower than of control sample (Fig. 2). In both packing conditions, the reduction of LAB of bacteriocins mixed meatballs was in consistent with other studies (Aymerich, Garriga, Costa, Monfort & Hugas, 2002, Coventry, Muirhead & Hickey, 1995). It was obvious that bacteriocins added meatballs and packed in vacuum condition could be kept longer than 21 days at 4 °C with total LAB counts of 4.18, 4.22 and 4.12 log cfu/g for the meatballs mixed with CCB, FDCB and nisin, respectively (Fig. 2B). However, a shorter storage time of 18 days for the meatballs packed in aerobic condition was found with 5.29, 4.22 and 4.16 log cfu/g when mixed with CCB, FDCB and nisin, respectively (Fig. 2A).

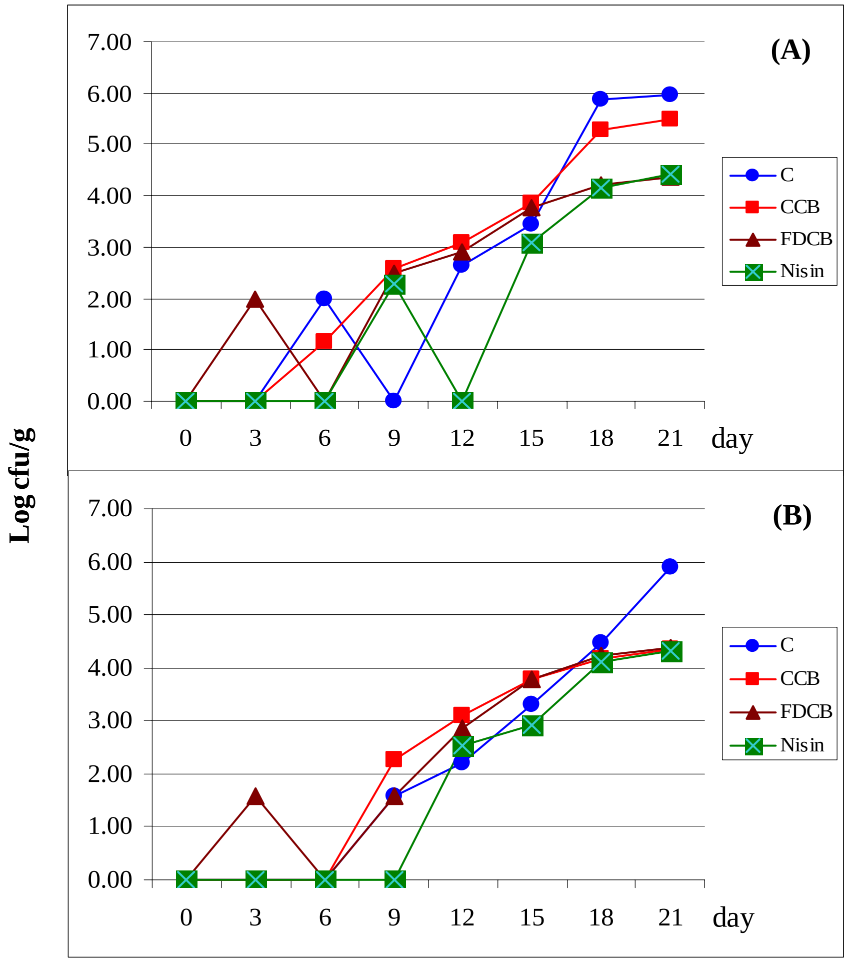

Fig. 2. Total lactic acid bacteria contents of chicken meatballs mixed with bacteriocins, stored at 4 °C. Abbreviations are the same as in Fig. 1.

pH of chicken meatballs. pHs of chicken meatballs are shown in Table 1. Throughout storage time, slight pH changes were observed for the meatballs mixed with either CCB and FDCB or nisin. In additon, pHs of FDCB and nisin mixed meatballs were lower ($p<0.05$) than those of CCB and control meatballs. Overall, the bacteriocin treated chicken meatballs packed in both conditions had pH values in the range of 5.99-6.26 while those without were about 6.22-6.30.

According to the Thai FDA microbial standard of ≤ 5.0 log cfu/g food sample, it could be mentioned that chicken meatballs mixed with crude bacteriocins obtained from *Lc. lactis* TISTR 1401, stored at 4 °C could be kept for about 18-21 and more than 21 days in aerobic and vacuum packaging, respectively. The antimicrobial effectiveness of FDCB was found comparable to that of commercial nisin and slightly better ($p<0.5$) than that of CCB. The pH values of FDCB and nisin added chicken meatballs were also lower than that of CCB and control meatballs.

Table 1. Mean pH values of chicken meatballs mixed with concentrated and freeze dried crude bacteriocins as compared with commercial nisin and control meatballs, stored at 4°C

Storage time (days)	Aerobic Packaging				Vacuum Packaging			
	C	CCB	FDCB	Nisin	C	CCB	FDCB	Nisin
0	6.27a	6.22b	6.19bc	6.16c	6.28a	6.24a	6.18c	6.17c
3	6.28a	6.24b	6.20bc	6.17c	6.30a	6.26b	6.21c	6.18c
6	6.27a	6.23bc	6.18c	6.16c	6.28a	6.24b	6.20bc	6.16c
9	6.26a	6.22b	6.18bc	6.16c	6.28a	6.23b	6.19bc	6.16c
12	6.27a	6.22b	6.23ba	6.16c	6.27a	6.23b	6.20bc	6.17c
15	6.26a	6.23a	6.18b	6.17b	6.26a	6.24a	6.21ab	6.18b
18	6.27a	6.22b	6.18c	6.16c	6.22a	6.25a	5.99b	6.17a
21	6.26a	6.25a	6.20b	6.17c	6.26a	6.24a	6.17b	6.18b

Different letters in the same day of storage are significantly different ($p < 0.05$), n = 6.
Abbreviations are the same as in Fig. 1.

IV. CONCLUSIONS

The most dominant bacterial strain from chicken meatballs in-house prepared was identified as *Bacillus* spp. Crude bacteriocins produced from *Lactococcus lactis* TISTR 1401 and prepared in the forms of 10-fold concentrated (CCB) and freeze dried powder (FDCB) were proved to have strong inhibition against this dominant strain. The uses of CCB and FDCB by mixing in chicken meatballs could prolong the product shelf life, stored at 4 °C, to 18-21 days for aerobically packed and longer than 21 days for vacuum packed condition while meatballs without crude bacteriocins could be stored only about 15 and 18 days, respectively. The effectiveness of CCB and FDCB to extend the shelf life of chicken meatballs in this study were comparable to that of commercial nisin.

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