The use of crude bacteriocins from *Lactococcus lactis* TISTR 1401 as biopreservative to extend shelf life of aerobically packed pork meatballs

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Abstract

The objective of this study was to investigate the use of crude bacteriocins as biopreservative to extend shelf life of aerobically packed pork meatballs. Crude bacteriocin supernatant (CBS) produced by *Lactococcus lactis* TISTR 1401 was coated on surface of pork meatballs in comparison with untreated ones, aerobically packed, and then stored in a cold room at 4 ^{o}C for 12 days. Total bacterial counts were observed on day 3 and day 9 for untreated and CBS treated meatballs, respectively. At day 12, the bacterial cell count of untreated sample was about 2 log cycles higher than that of CBS treated sample. The cell counts of *Enterobacteriaceae*, *Pseudomonas* sp. and LAB were observed between day 9 and day 12 while no *Brochothrix thermosphacta* growth was detected. Color measurements in L, a, b Hunter color system, no significant differences were found for the L and a values in both treatments during storage. However, the pork meatballs coated with CBS showed higher b values ($P < 0.05$) than did control samples. From sensory evaluation by QAD method, changes of color, water purge, slime, overall appearance, spoiled and oxidized odor. and overall acceptance of both meatball treatments were not observed while significant differences ($p < 0.05$) were found for abnormal odor.

Introduction

Lactic acid bacteria (LAB) have been used as microbial preservative for thousand of years in order to extend the shelf life of foods. Bacteriocins produced by Lactococcus species, especially nisin, are widely used in foods since the LAB have generally been regarded as safe (GRAS) organisms. Nisin has been shown to effectively inhibit the growth of Gram-positive bacteria, including many important foodborne pathogens. However, nisin is not widely used in meat products, because of binding of nisin to meat particles in meat systems (Deegan et al., 2006; O'keeffe and Hill, 1999). Since less activity of bacteriocins in meat products is expected due to binding of bacteriocin peptide with meat proteins during heat process. Therefore, application of bacteriocins onto surface of cooked meat products should be an alternative mean of application.

Pork meatballs are one of the economically important meat products with a high consumption in Thailand. In general, the pork meatballs are aerobically packed in plastic bags for sale at lower level market while those vacuum packed are sold in supermarket. In meatball processing, tapioca flour is usually used to increase water holding capacity and also increase production yield. This flour is normally contaminated with a large number of Bacillus spp. From our previous study, Bacillus spp. has shown to be most sensitive to bacteriocins produced by Lc. lactis TISTR 1401. In this investigation, therefore other bacterial species which could be responsible for the spoilage of pork meatballs include LAB, Psuedomonas, Enterobacteriaceae and Brochothrix thermosphacta were monitored during the storage of aerobically packed pork meatballs at 4 oC.

Materials and methods

Preparation of Bacteriocins. Bacteriocins were produced by the selected LAB strain of *Lc. 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 ^{o}C and controlled pH at 6.5. After fermentation, the broth was centrifuged at 12,000xg at 4 ^{o}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 ^{o}C for 20 min, cooled and stored at 4 ^{o}C until use.

Meatball manufacture. Pork meatballs were made from the meat batter containing 10 kg lean pork, 2% salt, 0.4% sodium phosphate, 15% ice and 4% tapioca starch. The batter was formed into meatballs in 60 ^{o}C water for 5 min then in 80 ^{o}C water for 20 min and dried at room temperature for 10 min. The meatballs were coated with crude bacteriocin supernatant (CBS) and cooled in a cold room (4 ± 1 ^{o}C) for 10 min. The CBS coated meatballs, along with uncoated ones were then separately packed in polyethylene bags and stored in a cold room at 4 ^{o}C.

Microbiological analysis. The meatball samples were taken every 3 days for analyses for a period of 12 days. A composite sample of 25 g was homogenized with 0.1% peptone water in a stomacher for 1 min. The serial dilutions were plated on PCA agar for total aerobic plate count and incubated at 25 ^{o}C for 48 h, MRS agar for LAB and incubated at 35 ^{o}C for 48 h, *Pseudomonas isolation agar for Pseudomonas* and incubated at 20 ^{o}C for 48 h, streptomycin sulfate thallous acetate actidione agar for *Br. thermosphacta* and incubated at 25 ^{o}C for 48 h, and violet red bile agar with glucose for *Enterobacteriaceae* and incubated at 37 ^{o}C for 48 h.

Color measurement and sensory evaluation. Color of the meatballs were measured in triplicate per sample in terms of L, a, b Hunter color system. Sensory evaluation was performed for color, appearance, purge, slime, abnormal, oxidized and spoiled odor, and overall acceptance using the quantitative descriptive analysis method with unstructured line score of 10 cm from less to high intensity by 6 trained panelists.

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

Results and discussion

Antibacterial property of crude bacteriocins on pork meatballs. The initial microbial loads for each bacterial species enumerated of the meatball batter were 6.18, 4.98, 4.73, 5.52 and 3.59 log cfu/g for total viable count, *Enterobacteriaceae, Pseudomanas* spp., LAB and *Br. thermosphacta*, respectively. The efficacy of *Lc. lactis* TISTR 1401 crude bacteriocins on retarding bacterial growth on the surface of pork meatballs is shown in Table 1. At day 0, there were no total aerobic plate counts of bacteria observed for both control and CBS treated meatballs. This fact was also observed for other bacterial groups. This could be due to the fact that the meatballs were prepared with a heat treatment at the final temperature of 90 ^{o}C that guaranteed pasteurization effectively inactivating vegetative cells and then stored under conditions that would limit the bacterial growth. However, cross contamination from the environment (i.e. airborne or food handlers) or the survival of spores or resistant cells was possible, as it is in commercial operations. For this reason, the total bacterial growth was not observed till day 3, while the CBS treated meatballs still had no bacterial growth until day 9 of refrigerated storage with about 1 log cfu/g lower than that of the control meatballs. At day 9, for other bacterial groups enumerated, only *Enterobacteriaceae* was observed. From day 9 to day 12, the total bacterial cell counts of control meatball increased about 2 log cycles while only 1 log cycle was found for CBS treated one. This reduction of microbial counts in meat and products by bacteriocins produced by the LAB was in consistent with other studies (Aymerich et al., 2002; Coventry et al., 1995). Moreover, the total bacterial count of control meatballs was about 2 log cycles higher ($p < 0.05$). From this finding and according to the Thai FDA standard for microbial count of 1×10^5 cfu/g food sample, it could be suggested that CBS produced by *Lc. lactis* TISTR 1401 effectively inhibited bacterial growth and be able to extend shelf life of pork meatballs at least 3 days longer than those without bacteriocins treated and aerobically stored at 4 ^{o}C. In addition, there was no *Br. thermosphacta* growth observed throughout 12 days of storage which could be due to the high temperature of meatball preparation.

Table 1. Microbial count (log cfu/g) of aerobically packed pork meatballs during storage at 4 ^{o}C

Storage	Aerobic count		*Enterobacteriaceae*		*Pseudomonas sp.*		Lactic acid bacteria		*Br. thermosphacta*	
Day	Control	CBS	Control	CBS	Control	CBS	Control	CBS	Control	CBS
0	-	-	-	-	-	-	-	-	-	-
3	3.41	-	-	-	-	-	-	-	-	-
6	4.21	-	-	-	-	-	-	-	-	-
9	4.80	3.57	4.87	-	-	-	-	-	-	-
12	6.26a	4.30b	5.28	4.22	-	4.23	4.72	4.25	-	-

Different letters in the same day of storage, within each bacterial count are significantly different ($P < 0.05$).

Color characteristics of pork meatballs. All color coordinates of the meatballs are shown in Table 2. Lightness (L values) of all meatballs slightly increased with the storage time but significant difference was not observed at the same day of storage. On the contrary, redness (a values) of all meatballs slightly decreased as the storage time progressed with no significant difference was found. However, the a value of CBS treated meatballs changed more slowly which could be due to the residual color of culture media on the meatball surface. Similarly, yellowness (b values) of all meatballs slightly decreased as the time of storage progressed with significant difference ($p < 0.05$) was found only at day 3. Although significant difference was not found at the same day of storage, the yellowness of CBS treated meatballs were slightly higher because of the color of culture media.

Table 2. Color coordination of aerobically packed pork meatballs during storage at 4 °C

Storage	L-value		a-value		b-value	
Day	Control	CBS	Control	CBS	Control	CBS
0	76.30	75.75	2.77	3.05	7.77	9.34
3	76.77	75.79	2.82	2.88	6.93b	8.72a
6	77.35	76.69	2.57	2.71	7.25	8.98
9	77.25	75.95	1.98	2.60	7.48	8.60
12	77.59	76.75	1.86	2.58	7.51	8.74

Different letters in the same day of storage, within each color value are significantly different ($P < 0.05$).

Sensory characteristics of pork meatballs. From the eight sensory attributes evaluated, only abnormal odor revealed differences ($p < 0.05$). Figure 1 shows the information of sensory evaluated in day 0, 6 and 12. It was obvious that the culture media caused higher abnormal odor score of the CBS treated meatballs. However, both CBS treated and control meatballs were equally accepted by the panelists throughout storage time. In addition, the oxidized odor of control meatballs was stronger than those of treated meatballs. This could be either oxidation processed more rapidly in control samples or the flavor of the culture media masked the oxidized odor of treated meatballs. It is suggested that further study should be made weather the bacteriocins have ability to retard oxidation of cooked meat products.

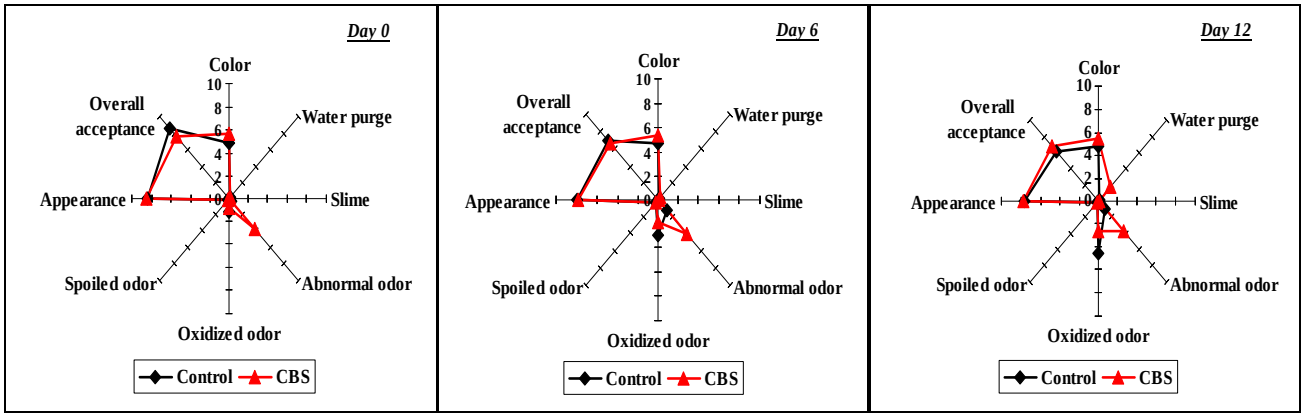

Figure 1. Sensory characteristics of aerobically packed pork meatballs, stored for 12 days at 4 °C.

Conclusions

Crude bacteriocins produced by *Lactococcus lactis* TISTR 1401 was successfully applied to extend the shelf life of pork meatballs. By surface application of crude bacteriocins on the meatballs, aerobically packed and stored at 4 °C, the shelf life of pork meatballs could be extended at least 3 days longer than those of control meatballs. Although color coordination (L, a, and b values) of the meatballs were not statistically differed, the culture media provided the meatballs with slightly darker in color. In addition, only abnormal odor attribute was significantly different ($p < 0.05$) and both CBS treated and control meatballs were equally accepted by trained panelists.

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