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Application of *Latilactobacillus curvatus* into Pickled Shrimp (*Litopenaeus Vannamei*)

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Abstract

Latilactobacillus curvatus **has a strong carbohydrate fermentative ability and antibacterial ability. It is considered as a promising probiotic by its excellent fermentation attributes and health advantages. Pickled shrimp derived from the fermentation process is highly appreciated by its unique texture, taste and flavor. However, this product is easily decomposed by spoilage bacteria especially *Staphylococcus.* This research evaluated the inoculation of L. curvatus (0.1-0.5 %) and different fermentation temperatures (28-30°C) on the reduction of *Staphylococcus aureus,* pH and overall acceptance of the pickled shrimp after 6 weeks of fermentation. Results showed that the fermentation process should be conducted at 29°C with 0.3 % *Latilactobacillus curvatus* (at initial density 9 log cfu/ml) to reduce pH to 3.70, completely against *Staphylococcus aureus,* obtain the highest sensory score (8.91).**

Keywords: *Fermentation, Latilactobacillus curvatus,* overall acceptance, pH, pickled shrimp, *Staphylococcus aureus*

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INTRODUCTION

The FAO/WHO definition of a probiotic—"live microorganisms which when administered in adequate amounts confer a health benefit on the host"—was reinforced as relevant and sufficiently accommodating for current and anticipated applications[1]. Health Canada has accepted *Bifidobacterium* and *Lactobacillus* in food at a level of 9 log CFU/g[2]. European Union countries suggest the utilization of specific species for nutrition and health advantages[3]. The Italian Ministry of Health has regulated the application of probiotic bacteria in the food industry under several terms, including a minimum number of viable cells (9 log CFU) administered per day, a full genetic characterization of the probiotic strain and a demonstratable history of safe use in the Italian market[4]. Overall benefit of probiotic was a supporting on healthy digestive tract against infectious diarrhoea, antibiotic-associated diarrhoea, gut transit, abdominal pain and bloating, ulcerative colitis and necrotizing enterocolitis[5-9]. The major benefits of probiotic were improvement of healthy immune system, reproductive tract, oral cavity, lungs, skin and gut–brain axis[10-11]. Probiotics inhibited prospective pathogens or released helpful metabolites/ enzymes to improve intestinal or extraintestinal immune effects[12-13]. *Latilactobacillus curvatus* shows milky white, translucent, and smooth colonies. It's a candidate probiotic included in the list of recommended biological agents for certification by the European Food Safety Authority. It had perfect fermentation characteristics and health advantages[14]. It is unique by its bacteriocinogenic property against pathogenic and spoilage bacteria especially *Listeria monocytogenes* and *Staphylococcus aureus, Bacillus cereus*, and *Enterococcus faecium*[15-18]. Bacteriocin extracted from *Latilactobacillus curvatus* can be utilized to coat on polyethylene film as positive product coating[19-20]. Organic acids were also derived from *Latilactobacillus curvatus*. These organic acids were responsible for pH reduction and fatty acid hydrolization to impart desirable flavor and aroma during sausage production[21-22]. *Latilactobacillus curvatus* is considered as beneficial probiotic to relieve obesity and hyperlipidemia by regulating the colon micro-system through attending with nutritional components or converting antimicrobial amino acids, retarding adipocyte differentiation and lowering fat accumulation via discharging of cholesterol and coprostanol via cholesterol metabolism[23-24]. *Latilactobacillus curvatus* is commonly isolated from fermented vegetable, beef, fish[25-28]. It's able to metabolize different carbohydrates like sucrose, glucose, trehalose, lactose, galactose, cellobiose and esculine[29-31]. Moreover, *Latilactobacillus curvatus* is *also* able to catabolize ascorbic acid, alcohol[32]. *Latilactobacillus curvatus* has ability to create tissue cantons via inter-binding to establish in the colon route contributing to probiotic role by interfering with harmful microorganisms to support the owner and retard the foodborne bacteria[33-34]. Hydrophobicity is a decisive variable affecting cell adhesion. *Latilactobacillus curvatus* has peptidoglycan layer of the cell partition to overcome high concentration of lysozyme in saliva, low pH in stomach and bile in the upper intestine by altering the profile and absorbent of the cell lining or forming outer polysaccharides[35-41].

White leg shrimp (*Litopenaeus vannamei*) is one important seafood in the world. It has great nutritional values because it contains an excellent source of proteins, minerals, polyunsaturated fatty acid content, but low fat, less cholesterol[42-43]. White shrimp can be fermented into value-added product like pickle. Pickled shrimp is highly appreciated by its specific texture, taste and flavor[44]. Pickled shrimp has much more amino acid, glycogen and mineral compared to pickled vegetable[45-47]. *Staphylococcus* was identified as the main spoilage bacteria in decomposition of pickled shrimp[48-49]. Purpose of our research was to investigate the impact of several ratios of *L. curvatus* as starter culture and different fermentation temperature on the elimination of pathogenic bacteria (*Staphylococcus aureus*), pH and overall acceptance of the pickled shrimp after 6 weeks of fermentation.

MATERIAL AND METHOD

Material

White shrimps (*Litopenaeus vannamei)* were purchased from local market in Bac Lieu, Vietnam. They were temporarily preserved in flake ice ready for experiments. Besides white shrimp, this research also used other ingredients such as sodium chloride, calcium chloride, saccharose, ethanol, galanga, plastic jar. *Latilactobacillus curvatus* and *Staphylococcus aureus* were supplied from Rainbow Trading Co. Ltd, Vietnam.

Researching method

White shrimps were rinsed with clean water before mixing with 13.5% of NaCl, 1.5% of $CaCl_2$, 6.5% of sucrose, 5% of ethanol, 10% sliced galangal. *Staphylococcus aureus* was inoculated into shrimp mixture at 0.05 % with the initial density 9 log cfu/g. *Latilactobacillus curvatus* was activated before experiments. Stock density of *Latilactobacillus curvatus* was enumerated at 9 log cfu/ml. *Latilactobacillus curvatus* was inoculated into shrimp mixture at different ratios (0.1-0.5 %). The fermentation temperature was conducted in range (28-30 °C) for 6 weeks. Samples were taken to evaluate *Latilactobacillus curvatus* colony survival, pH and overall acceptance. This sampling lasted until the 6th week. *Latilactobacillus curvatus* enumeration was performed by method described by Van Reckem et al[50]. 5 gram of pickled shrimp was injected into a mixing pouch with diluents in ratio 1:10 and 0.1% peptone. This mixture was homogenized by stomacher for 1.5 minutes and adequate dissolving in buffer solution were prepared, cover on MRS film. MRS film was kept at 29±1 ◦C for 72 h. *Latilactobacillus curvatus* density (cfu/g) was enumerated by colony counter. *Staphylococcus aureus* was enumerated by Petrifilm plate. pH of samples was evaluated by pH meter. Overall acceptance (sensory evaluation) was examined by a panel of specialists basing on 9-point Hedonic scale ranging from 1 = Dislike extremely and 9 = Like extremely. The hedonic scale admitted that specialists' interests exist on a constant and that their feedbacks could be classified into like and dislike.

Statistical analysis

All testings were set in 3 replications. The data were expressed as mean±standard deviation. Statistical parsing was based on the Statgraphics Centurion software version XVI.

Table 1. Survival of *Staphylococcus aureus* (log cfu/g) in the pickled shrimp affected by inoculation ratio (%) of *Latilactobacillus curvatus* (9 log cfu/ml) and fermentation temperature (oC) after 6 weeks of fermentation

Fermentation temp. (°C)	Inoculation ratio (%) of *Latilactobacillus curvatus* (9 log cfu/ml)				
	0.1	0.2	0.3	0.4	0.5
28.0	4.21±0.02^{a}	2.85±0.00^{ab}	1.15±0.01^{b}	0.66±0.00^{bc}	0.25±0.02^{c}
28.5	2.65±0.01^{a}	1.32±0.03^{ab}	0.63±0.02^{b}	0.21±0.01^{c}	0.12±0.03^{c}
29.0	1.04±0.00^{a}	0.57±0.02^{b}	0.00±0.00^{c}	0±0.00^{c}	0±0.00^{c}
29.5	1.93±0.03^{a}	1.26±0.01^{ab}	0.29±0.03^{b}	0.12±0.02^{bc}	0.07±0.01^{c}
30.0	3.41±0.02^{a}	1.94±0.00^{ab}	0.89±0.01^{b}	0.53±0.03^{bc}	0.20±0.02^{c}

Note: the numbers were presented as the mean of 3 samples; the same symbol was considered insignificant difference (α = 5%).

Table 2. pH of the pickled shrimp affected by inoculation ratio (%) of *Latilactobacillus curvatus* (9 log cfu/ml) and fermentation temperature (°C) after 6 weeks of fermentation

Fermentation temp. (°C)	Inoculation ratio (%) of *Latilactobacillus curvatus* (9 log cfu/ml)				
	0.1	0.2	0.3	0.4	0.5
28.0	4.67±0.00^{a}	4.46±0.02^{ab}	4.31±0.00^{b}	4.22±0.03^{bc}	4.13±0.01^{c}
28.5	4.11±0.03^{a}	4.02±0.01^{ab}	3.91±0.00^{b}	3.83±0.02^{bc}	3.74±0.00^{c}
29.0	3.96±0.01^{a}	3.81±0.00^{b}	3.70±0.02^{c}	3.59±0.00^{c}	3.50±0.03^{c}
29.5	4.05±0.02^{a}	3.97±0.03^{ab}	3.84±0.01^{b}	3.71±0.01^{bc}	6.62±0.02^{c}
30.0	4.33±0.00^{a}	4.15±0.01^{ab}	4.08±0.00^{b}	3.99±0.02^{bc}	3.85±0.01^{c}

Note: the numbers were presented as the mean of 3 samples; the same symbol was considered insignificant difference (α = 5%).

RESULT AND DISCUSSION

Fermentation was a useful preservative method to prolong the seafood stability for long-term consumption[51]. It was prepared by mixing raw material with salt, keeping at room condition[52]. Table 1 showed the survival of *Staphylococcus aureus* (log cfu/g) in the pickled shrimp affected by inoculation ratio (0.1-0.5 %) of *Latilactobacillus curvatus* (9 log cfu/ml) and fermentation temperature (28-30 °C) after 6 weeks of fermentation. It's obviously noticed that 0.3 % *Latilactobacillus curvatus* (at initial density 9 log cfu/ml) could effectively suppress *Staphylococcus aureus* growth (the highest 1.15±0.01 log cfu/ml at 28 °C to the lowest 0.00±0.00 log cfu/ml at 29 °C). 29 °C was identified as appropriate temperature for *Latilactobacillus curvatus* to proliferate against pathogenic and spoilage bacteria (the highest survival of *Staphylococcus aureus* 1.04±0.00 log cfu/ml at 0.1 % inoculum to the lowest survival of *Staphylococcus aureus* 0.00±0.00 log cfu/ml at 0.3 % inoculum). It has a distinct capacity to emit antibacteria substances with powerful antimicrobial activity against pathogen and spoilage bacteria in meat preservation. As a kicked microbial for fermented sausage, *L. curvatus* improves desirable flavor for the final product[53]. It can decrease the load of *L. monocytogenes*, a main pathogen in fermented sausages[54]. Moreover, it also greatly retard the proliferation of the harmful bacteria like *Enterobacteriaceae, Pseudomonas fragi*, *Pseudomonas putida, Brochothrix thermosphacta*[55].

Table 2 presented pH reduction in the pickled shrimp affected by inoculation ratio (0.1-0.5 %) of *Latilactobacillus curvatus* (9 log cfu/ml) and fermentation temperature (28-30 °C) after 6 weeks of fermentation. There was a down trend of pH by increasing inoculation ratio of *Latilactobacillus curvatus* and fermentation temperature. The highest fermentation efficiency recorded at 29°C, pH of the fermentation batch decreased from 3.96±0.01 to 3.50±0.03. *Latilactobacillus curvatus* had a strong ability to ferment different carbohydrates to form organic acids[29-31]. Organic acids were produced from *Latilactobacillus curvatus* to be used in pH reduction and fatty acid hydrolization to enhance desirable flavor and aroma[21-22].

Fig. 1 revealed the overall acceptance of the pickled shrimp affected by inoculation ratio (0.1-0.5 %) of *Latilactobacillus curvatus* (9 log cfu/ml) and fermentation temperature (28-30°C) after 6 weeks of fermentation. The fermentation process should be conducted at 29 °C with 0.3 % *Latilactobacillus curvatus* (at initial density 9 log cfu/ml) to achieve the highest sensory score (8.91±0.04). Meanwhile the lowest overall acceptance (6.41±0.05) was noticed at 28 °C with 0.1 % inoculum. *L. curvatus* can metabolize nitrosamines and fatty acids via different dedicated enzymatic systems[56-58]. *L. curvatus* could decompose sarcoplasmic protein to release peptides and amino acids[59]. In aging, these peptides and amino acids straight forward improve sensory attributes of final

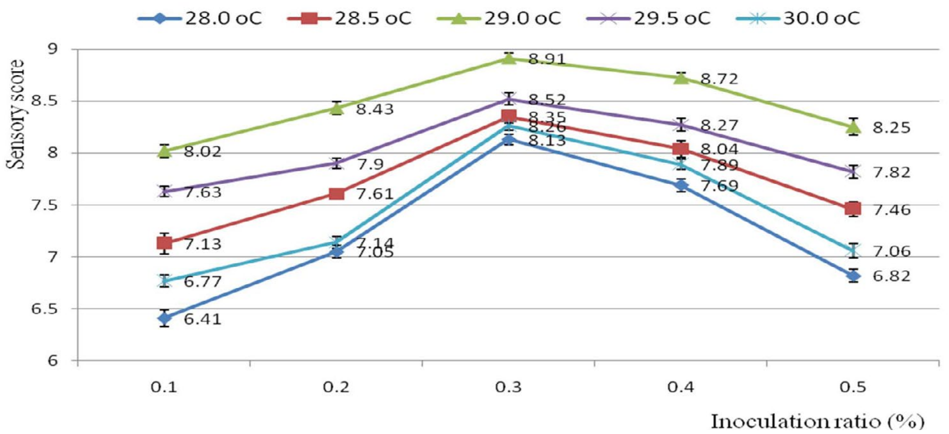

Fig. 1. Overall acceptance of the pickled shrimp affected by inoculation ratio (%) of *Latilactobacillus curvatus* (9 log cfu/ml) and fermentation temperature (°C) after 6 weeks of fermentation.

products[60]. Short-chain and medium-chain free fatty acids also released from hydrolyzing esters by *L. curvatus.* These fatty acids contribute to improvement of sensory characteristics of the sausage. According to Nguyen et al., white shrimp should be fermented at 28°C for 28 days to get a pleasant taste[44]. A supplementation of garlic into pickled white shrimp was reported[61]. In pickling, a great amount of unique amino acids was released. Pickled product was safe and stable over 6 months at ambient condition[62] (Chandrashekhar, 1979). Pasteurized marinated shrimp in green curry paste was safe for 15 days at 0-3°C[63].

CONCLUSION

Latilactobacillus curvatus is a promising starter culture recommended for meat processing industry. It can utilize carbohydrate for fermentation. It exhibits bioprotective properties by releasing bacteriocin against harmful microorganism. This bacteria has numerous adoptions in seafood preservation and in human wellness improvement. This research has successfully demonstrated the influence of different ratio of *L. curvatus* as starter culture and various fermentation temperature on the reduction of pathogenic bacteria (*Staphylococcus aureus*), pH and overall acceptance of the pickled shrimp after 6 weeks of fermentation.

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DATA AVAILABILITY

All data sets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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