

Abstract of Dissertation

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Title : Study of foodborne disease: Hygienic status and prevalence of food-borne pathogens in domestic natural cheeses produced in Hokkaido, Japan

(北海道産ナチュラルチーズの食品安全性に関する研究)

Abstract:

Natural cheese is made from raw or pasteurized milk and it is widely consumed around the world. Despite its wide popularity, sporadic foodborne outbreaks caused by *Salmonella* spp. *Listeria monocytogenes*, pathogenic *Escherichia coli* and *Staphylococcus aureus* are reported and linked with consumption of contaminated cheese. In Japan, foodborne disease related to the consumption of natural cheese is not common. This could relate to the good hygienic practice and low level of cheese consumption as compared to other countries. However, a recent report from Hokkaido showed the incidence of foodborne disease outbreak of *L. monocytogenes* caused due to the consumption of contaminated cheese. Moreover, another report also showed prevalence of *S. aureus* in cheese while some of the isolates contain enterotoxin genes. Except those limited reports, however, the hygienic status and prevalence of these foodborne pathogens in domestic natural cheese produced in Hokkaido is not known. The objectives of this dissertation were, therefore, to identify the hygienic status and microbial quality of domestic natural cheese; to investigate prevalence of foodborne pathogens in domestic natural cheese produced in Hokkaido with particular focus on *L. monocytogenes*, *Salmonella*, pathogenic *E. coli*, and *S. aureus*; and to characterize the virulence and antimicrobial resistance properties of *S. aureus* isolates obtained from domestic natural cheese produced in Hokkaido.

The first chapter describes hygienic status and microbial quality of domestic natural cheese produced in Hokkaido is at its fine status. A total of 200 natural cheese samples were collected and used for this study. A standard plate count, coliform count and spore formers counts were conducted following standard methods of FDA and MHLW to assess the

hygienic status. The result of the standard plate count ranged between 10 cfu/g and  $1.35 \times 10^9$  cfu/g. Among the samples, most of them (75.5%) had counts ranging between  $10^5$  cfu/g and  $10^9$  cfu/g. The overall spore former count result showed the minimum and maximum spore former counts of 10 cfu/g and  $5.2 \times 10^5$  cfu/g, respectively. For coliform count, 78% of the samples had counts below detection limit. The minimum and maximum counts of coliform were 10 cfu/g and  $6.3 \times 10^7$  cfu/g respectively. Among the coliform positive samples, soft cheese tend to have more coliform positivity than others where 29%, 17%, and 13% of soft, semi-hard and hard cheeses respectively were positive for coliform count. In addition to the cheese type, samples in different packages also had varying results where those in paper package had more coliform positivity. Absence of coliform in 78% of the samples indicates good hygienic quality of domestic natural cheese. The occurrence of coliform in 22% of the samples, however, remind the need to maintain the existing best production hygiene work to improve more.

The second chapter describes the prevalence of foodborne pathogens including *L. monocytogenes*, *Salmonella*, Pathogenic *E. coli* and *S. aureus* using standard methods of ISO and FDA. Moreover, PCR based investigations and MALDI-TOF MS methods were also employed for identification of presumptive pathogens. The results demonstrated that *L. monocytogenes*, *Salmonella* and pathogenic *E. coli* were not prevalent in a total of 126 samples inspected. The MALDI-TOF MS result for the suspected samples of *Listeria* showed that the isolates of those colonies were neither *L. monocytogenes* nor other *Listeria* Spp. One of the samples on the other hand (0.80%) showed positive PCR amplification for *ipaH* gene indicating possible contamination of enteroinvasive *E. coli* or *Shigella* spp. in this product. Yet neither of the pathogens were detected by culture method indicating that these pathogens are not prevalent in domestic natural cheese. On the other hand, 50% of the 74 natural cheese samples showed positivity for *Staphylococcus* spp. while some of the samples contain the counts exceeding  $10^5$  cfu/g indicating presence of some inappropriate handling practice. However, *S. aureus* was detected only in 4% of the samples. The result showed absence of common foodborne pathogens in domestic natural cheese indicating its microbiological quality and safety.

The third chapter described characterization of enterotoxin genes and antibiotic resistance properties of *S. aureus* isolates. PCR method was used to detect staphylococcal enterotoxins using their specific genetic markers. The result showed that some of the isolates had genes for enterotoxins A and H (*sea* and *seh*). Among all the isolates screened for the antibiotics resistance, none of them was resistant to oxacillin indicating no methicillin resistant strains. Moreover, all of the isolates tested were susceptible to tobramycin, fosfomycin, minocycline, imipenem and vancomycin. Furthermore, the result also showed that 12.5% of the isolates were susceptible to all the antibiotics tested. On the other hand, the results also showed that a total of 62.5% of the isolates were resistant to penicillin. In addition, 25% of the isolates were resistant to streptomycin and 12.5% isolates had an intermediate response to amikacin. The presence of genes for enterotoxin A and H (*sea* and

seh) among the *S. aureus* isolates indicate potential concern for the health of consumers due to staphylococcal food poisoning and the need to improve hygienic practice to prevent contamination.

In conclusion, most of the samples appeared negative for coliform count indicating good hygienic status. Among the samples analyzed, 78% of them comply with US ordinance for grade A milk and products and 92% of the samples also comply the standard of European commission for coliform count in natural cheese. Yet some samples had high coliform counts indicating possibility of cross contamination after cheese making process or failure in pasteurization process. This indicates the need for improvement. The absence of *Salmonella*, *L. monocytogenes* and pathogenic *E. coli* also indicate the higher safety and quality of domestic natural cheese produced in Hokkaido. However, detection of *S. aureus* in three samples (4%, n=74) also shows possible post processing contamination and the need for improvement of production hygiene. Especially, some of the *S. aureus* isolates had enterotoxin genes that are responsible for food poisoning outbreaks both in Japan and elsewhere. The occurrence of enterotoxigenic strains leaves the potential risk on the health of consumers related to the consumption of contaminated cheese and indicate the need to further improve the existing best production hygiene.

Notes 1. Fill in the Japanese translation for an English in the ( ).

2. Abstract should be between 1,800 and 2,200 characters in Japanese, or be between 1,000 and 1,400 words in English.
3. Do not include figures and tables.
4. Abstract can be longer than one page.