CHAPTER V

DISCUSSION AND CONCLUSIONS

Average total colony formation of the sausage samples was 3.38 ± 0.27 log cfu/gram. Twenty percent of all samples did not achieve the Thai Community Product standard. The percentage of the sausage samples which did not achieve the standard rose from 5% in the morning to 33% in the evening. The variation of sausage internal temperature in the morning period samples was higher than in the afternoon and evening samples. It may be that cooking had been done primarily in the morning and that the sausage internal temperature may be regarded as a key factor in reducing total colony counts in sausage. In a previous study of Northern Thai sausage, the total colony count numbers significantly increased when samples were kept at 20^0C-26^0C for 4 days in both the control group and the preservative substance application group (Rujanakraikarn et al., 1989). The internal temperature of the sausage samples in this study was within the range which allows mesophillic bacteria to grow. Many foodborne pathogens such as *Salmonella* spp., *Staphylococcus aureus,* and *Clostridium perfringens* are mesophillic bacteria (Adams and Moss, 2008). In this study, the average internal temperature was lower in the evening samples. Producers should routinely check the internal temperature of the sausage after cooking and control the duration of cooking. Such measures could decrease the initial microbial load in the product. The core temperature should reach 78^0C and the duration of cooking should be 1 minute of heat treatment per 1 mm of sausage diameter (Heinz and Hautzinger, 2007). In order to safely keep the sausages after cooking, they must always be stored at a temperature of not more than 4^0C (Heinz and Hautzinger, 2007). The percentage of sausage samples that had growth factors in the same growth range of microorganisms demonstrated that the growth factors in the Northern Thai sausage were able to support growth of mesophillic bacteria.

Average yeast and mold count was 2.00 ± 0.09 log cfu/gram. Twenty-eight percent of the sausage samples did not achieved the Thai Community Product standard. The percentages of the sausage samples below the standard were 30%, 20%, and 33% for the morning, afternoon, and evening samples, respectively. In comparison with the growth factors in the sausage, the mean temperature in the evening was in the growth range required by yeasts and molds. Even at lower temperatures, yeast and mold are capable of growing during storage periods. A previous study indicates that Northern Thai sausage after cooking at between 25^0C and 30^0C can deteriorate from colonization by molds after 8 days of storage (Rujanakraikarn et al., 1989). Refrigeration can reduce temperatures to a minimum of 4^0C. At that temperature, yeast and mold (psychrotropic microorganisms) may still grow. The minimum growth temperature for psychrotropic microorganisms ranges between -5^0C to 5^0C (Adams and Moss, 2008). Freezing would be a suitable method for storing cooked sausage, even though Northern Thai sausages are a ready to eat food. Moreover, consumers can visually observe spoilage of mold. In another study, of 150 samples of spice used in making sausage, 7% had more than 100 cfu/gram of mold and 83% had more than 100 cfu/gram of yeast species (De Boer, 1983). In the present study, all sausage samples had less than 100 cfu/gram of yeast. The concerning of mlod contamination in food refer to thier mycotoxin which can not destroyed by cooking temperature. It has been demonstrated that the use of spices contaminated with toxigenic mold strains lead to a secondary contamination of the final product with aflatoxins (Guergue and Ramirez, 1997; Aziz and Youssef, 1991). The raw material quality controling was important to prevent mycotoxin contamination in the sausage production. The level of mycotoxin in Northern Thai sausage still needs further study.

The presence of *Escherlichia coli* indicates fecal contamination (Suwansonthichai and Rengpipat, 2003). Poor hand hygiene is indicated by high levels of *Escherlichia coli* (Shah et al., 2009). However, *Escherichia coli* was not founded in this study. From a previous study of 126 fresh raw Italian sausage samples, which is the same type as Northern Thai sausage, Verocytotoxigenic *Escherichia coli* (VTEC) were found in 16% of the samples (Villani et al., 2005). The primary cause of *Escherlichia coli* outbreaks is a failure to cook products

adequately. The internal temperature is critical to decreasing number of *Escherlichia coli* in the cooked sausage. *Escherlichia coli* is not a heat resistant bacteria, but it can survive refrigerated or frozen storage for extended periods (Adams & Moss, 2008). The refrigeration temperature for sausage should be kept at below 4.4^0C, while a freezer should be at least -17.8^0C (Manning, 2010).

Salmonella spp. was not founded in this study, so all sausage samples achieved the standard for *Salmonella* spp. Meat is supposed to be one of the primary vehicles for *Salmonella* spp. In another study, *Salmonella* spp. was identified in 55%, 70.5%, and 34.5% of freshly cut pork, transported pork, and pork retail products, respectively, in Chiang Mai, Thailand, (Sanguankiat et al., 2010). Salmonella outbreaks occur because undercooked meat or cross-contaminated foods are consumed without further cooking (Adams and Moss, 2008). Results of the present study indicate that the cooking process may play an important role in eliminating *Salmonella* from the product. The possibility of cross-contaminated from food handlers may bring the organism back and could cause food safety problem for consumers.

One sausage sample was positive for *Staphylococcus aureus* in this study. The averages of growth factors were in the same range as for *Staphylococcus aureus*. Their enterotoxin production ranges are show in Figure 15. Consumers should reheat cold stored sausage before consuming. The D-values for *staphylococcus aureus* at 65^0C were 0.2- 2.00 minutes (Heinz and Hautzinger, 2007). Most staphylococcal food poisoning results from improper food preparation or storage. *Staphylococcus aureus* is transferred into the food from the person preparing it (Ingraham and Ingraham, 2000). The presence of *Staphylococcus aureus* indicates contamination from skin, mouth, or nose of the food handler (Bennett, 2001). *Staphylococcus aureus* was the most frequently isolated from the interior of refrigerators. It was founded in 6.4 % of 342 refrigerators (Jackson et al., 2007). From study results, it may be concluded that sausages are cross-contaminated from the skin of food handlers or equipments. Enterotoxin of *S. aureus* is stable at a heat treatment at 100^0C for 30 min, a treatment that readily kills the microorganism (Bennett, 2005; Dinges et al., 2000). The good practices of food handlers, adequate core temperature

control of sausage, and regular cleaning of storage equipment are important methods for controlling *Staphylococcus aureus* contamination in sausage.

One sausage sample was positive for *Clostridium perfringens* in this study. *Clostridium perfringens* is widespread in nature and is normally found in the intestinal tract of animals and humans (Schothorst, 1999). *Clostridium perfringens* contamination reflects that animal carcasses and cuts of meat may become contaminated from soil, animal feces or handling during slaughtering and processing (Heredia and Labbe, 2001). In one study, of one hundred and fifty spices samples, 100% had *Clostridium perfringens,* but 98% of the positive samples had less than 100 cfu/gram (De Boer, 1983). In another study of 115 spice samples, 12.17% were positive for *Clostridium perfringens* (Aguilera et al., 2005). Contamination of raw materials is an important source of *Clostridium perfringens* in food. In this study, there was an increase in the number of samples where growth factors were in the optimum growth range of *Clostridium perfringens* as the collection time moved from morning to evening. From the study results, it can be concluded that there was contamination of raw materials. The microclimate in the sausage, the nearly anaerobic condition after stuffing. In this study, some producers keep the sausage in the vacuum package which an anaerobic condition in the package could support the growth of *Clostridium perfringens*. A good way to control sausage contamination problems is raw material quality control. The previous study in turkey meat recomended producer should cook turkey meat until internal temperature reach 74^0C to eliminated *Clostridium perfringens*. The another study on beef, beef roasts cooked in plastic bags in a water bath at 60-61^0C, holding the product to an internal temperature of 60^0C for at least 12 minutes can reduced the *Clostridium perfringens population* by about 3 log cycles (Jay, 2000). Some food-related *Clostridium perfringens* strains form clostridia spores that can survive most cooking temperatures (McCabe-Seller and Beattie, 2004). Consumers should adequately reheat cold stored sausage before consuming. The recommended temperature for reheating is above 60^0C (De Jong et al., 2004). *Clostridium perfringens* is classified into five toxinotypes (A, B, C, D, and E) based on the production of four major toxins (alpha, beta, epsilon, and iota toxins) but the enterotoxin of *Clostridium perfringens* type A is heat sensitive (biological activity destroyed at 60^0C for 10 min) (Jay, 2000).

Clostridium perfringens cannot multiply when material is stored at temperatures below 10^0C but spores of *Clostridium perfringens* are able to germinate at 3^0C and are able to germinate with and without heat-activation (De Jong et al., 2004). The suitable method to keep cooked sausage is freezing.

On the basis of these research findings, it can be concluded that the microclimate in Northern Thai sausage is suitable for the growth of some foodborne pathogens. Producers and consumers should be informed about preservation methods which decrease the risk of food spoilage and foodborne disease.