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Prediction on Microbiological Quality of Industrial Chicken Sausages during Distribution to Retailer vicinity Bangkok

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ABSTRACT

Predictive survival of microbiological quality of chicken sausages was simulated using ComBase® microbial predictive software. The prediction was based on real time and temperature monitoring chicken sausages during distribution to retailer around Bangkok area. Six pathogens, which were *Pseudomonas* spp., *Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes, Escherichia coli* and *Clostridium perfringens* were selected respectively for simulation during distribution. Five parameters were selected to predict the growth of selected pathogens during distribution period (maximum temperature from observation periods, pH, A_w, and initial log of Total Aerobic Plate Count (TAPC). The result showed that *Clostridium perfringens*, *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus*, *E.coli* and *pseudomonas* spp count ranged from 0.11 log CFU/g to 1 log CFU/g during distribution at 19.5°C for 9.05 hours. *Pseudomonas* spp showed the highest growth (1 log cfu/g) while *Clostridium perfringens* showed the lowest growth (0.11 log cfu/g) after distribution. As a conclusion, higher the temperature and longer the observation period could increase the growth rate of selected pathogens on distribution the products.

Keywords: Chicken sausages, temperature distribution, predictive analysis, microbiological quality, pathogens

INTRODUCTION

Strict temperature control in cold chain could minimize the risks of potential pathogenic bacteria. Sausages and other meat products are perishable in nature and require proper refrigeration temperature. These perishable and semi perishable foods are one of the fastest growing sectors for grocery in retail industry. Joshi, et., al, (2010) reported that, handling of perishables were more complicated and involved higher risks compared to non-perishable products due to their common nature of deterioration and limited shelf life. James (2008) reported that, from 1562 cases of food poisoning reported in the United Kingdom from 1986 to 1988, there were 970 cases and about (62%) occurring at home. Joshi et al. (2010) also reported that, the consumer link was the weakest link and easily broken in the food cold chain system. Maintaining low temperature of fresh produce or minimally processed product could decrease the level of microbial growth and delay deterioration of spoilage caused by bacteria in the cold chain. Rediers

et al. (2009) suggested that, to maintain the quality of minimally processed foods, the temperature should be kept lower or at least at 5°C to decrease the multiplication of spoilage microorganism and pathogenic bacteria. The researchers also found that maintaining the correct temperature throughout the supply chain could reduce microbial spoilage of endive. Temperature abuse had significance effect on coliform growth but did not increase the level of mesophilic bacteria. The level of all indicator microorganisms and pathogen was below the limit that was prescribed by European Ordination EC 2073/2005 (Rediers et al., 2009). Temperature reducing effect in chilled foods can slow down the deterioration process by extending the lag phase of microorganisms. The microbiological status of chilled foods has become more expressive and big issues in most countries. Food manufacturers and government agencies have to find the best way to control this microbiological issues and the traceability of the system should be required to resolve the issues in both parties (Walkers and Betts, 2000).

Cooked chicken sausage is made from red meat and white meat likely from beef or poultry such as chicken or the combination of the meat (FSIS, 2011). Normally the sausages are made from various of processes started from flaking, chopping of meats, mixing with different types of seasoning and stuffing into natural or synthetic casing and then cooking at high temperature either smoking or steaming. Cooked sausages are simple cooked or smoked has a soft texture and must be refrigerated (FSIS, 2011). Convenience and diversity of the products are the main reasons the sausages are commonly consumed nowadays. The sausage production is divided into several steps firstly by reducing the particle size of meat. After that, the minced meat is mixed with other ingredients and seasoning. Then, the paste was stuffed into specific casing and linked for specifically length and packaging finally (Youling and William, 2001). Dennis and Stringer (1992) reported that, the important criteria in storing perishable products like sausages are the temperature monitoring and control during processing until storage.

Temperature abuse is one of the factors leading to growth of pathogens in meat products that can cause food poisoning. The temperature of meat products like sausages must be maintained at -1 ± 0.5°C during storage and transportation to avoid the growth of bacteria. James (1996) reported that, all products that were kept in a container must be maintained at ± 1°C either in refrigerated or insulated container during distribution to retailer. Proper handling and extra care need to be taken in order to maintain the temperature during storage and transportation (James, 1996). Bear in mind, reducing the temperature of the sausages does not kill the potential microbes but could slow down the growth of some microbes in the system. Meat products are considering highly perishable foods and easily deteriorate if the temperature control and monitoring system is interrupted in the cold chain. Cold chain in the meat and poultry products such as sausages are directly interdependent operations in the production, storage, and distribution to consumer. For most cook-chill foods, the foods need to be cooked at 75°C and later cooled to below 20°C within 120 minutes and stored at 0°C to 4°C In conclusion, food safety and quality of sausage and meat products must be maintained throughout all the processes until the food is distributed.

Meats by products like sausages and frankfurter however, microbial responses to signify the traditional microbiological methods are normally not solely rely on intrinsic and extrinsic factors, such as temperature that only relevant to certain condition or parameter like curing ingredients in a fresh ham of limited example could be summarized as a simple predictive value (Baird Parker and Kilsby, 1987).P redictive food modeling (PFM) using mathematical models could solve the issues of food borne infection. Thus could prevent of food poisoning and intoxification incidence. In a study by Mc Millin, 1981 of sausages made from hot-boned pork, showed that increasing significantly higher counts of mesophiles but there is no significant differences in psychrotrophs were found in the product made from hot-boned pork than in the cold-boned product. There is no significant differences were found between this product and a cold-boned product relative to coliforms, staphylococci, psychrotrophs, and mesophiles. The predominant organisms, after storage, for both products were most likely enterococci and lactobacilli. In the case of pork sausages using natural casings have been shown to contain large numbers of bacteria. Riha and Solberg found the counts was ranged from $\log_{10}$ 4.48 to $\log_{10}$ 7.77 cfu/g and from $\log_{10}$ 5.26 to $\log_{10}$ 7.36 cfu/g for wet-packaged casings. More than 60% of the isolates from these natural casings consisted *of Bacillus* spp., followed by clostridia and pseudomonads. In other study

of slime from frankfurters, these investigators found that 275 of 353 isolates were bacteria, and 78 were yeast and the *B. thermosphacta*was the most conspicuous single isolate. With regard to the incidence of *C. botulinum* spores in liver sausage, 3 of 276 heated (75°C for 20 minutes) and 2 of 276 unheated commercial preparations contained type A botulinal toxin (Hauschild and Hilsheimer, 1983) The most probable number (MPN) of botulinal spores in this product was estimated to be 0.15/kg. FDA in the United States was examined of 32,800 packages of frankfurters and they found 532 (1.6%) and about 90% of all isolates were serotype 1 of L. *monocytogenes* .(Wallace et. al., 2003). A study of luncheon meats for *L. monocytogenes* in the states of Maryland and California, the organism was found in 82 of 9,199 (0.89%), (Gombas et. al., 2003).

An outbreak of *E. coli* 0157:H7 from dry cured salami happened in the states of California and Washington in 1994, involved 23 victims (CDC 1995). Following this outbreak, a series of studies were conducted on the conditions of pepperoni manufacture that are needed to effect a log 5 reduction in numbers of specific pathogens. By using a 5-strain cocktail of *E. coli* 0157:H7 at a level of $\geq 2 \times 10^7$/g, it was found that the non thermal process destroyed only about log 2 units/g and in order to reduce of log 5–6 reduction they are suggested post fermentation heating to an internal temperature of 63°C instantaneous for 60 minutes was needded (Hinkens et al., 1996). Another factors contribute to this evolving pattern of food borne disease was changes in food processing .There is increasing reliance on refrigeration and the cold chain as a way of extending the shelf life of fresh foods and this has contributed to the emergence of psychrotrophic pathogens such as *Listeria monocytogenes* as important concerns. For microbiological spoilage or quality of food, predictive microbiology is recognized as a scientific-based reliable tool for providing an estimation of the course of the bacteria in the foods, estimating shelf-life of the product in cases where the cause of food spoilage or unacceptability is known to be microbiological (Kilcast and Subramaniam, 2000; Pérez-Rodríguez and Valero, 2013).

Total viable counts (TVC) provide an information about the remaining shelf life of food products based on its quality and it cannot directly related towards a safety assessment but can be used as part of quality assessment (HPA, 2009; FSAI, 2014). However, high level of TVC indicates the level of microbial contamination which means there is a predominant organism present in the food which can translate to quality issues and possibly poor temperature control. Acceptability therefore depends on which organism predominates (HPA, 2009). Enterobacteriaceae and *E. coli* on the other hand are known to be hygiene indicator organisms and basically reflects the hygienic quality of the food. Enterobacteriaceae group includes pathogenic species e.g. *E.coli*, *Salmonella*, *Shigella*, and *Yersinia.*

In this study, the chicken sausages were surveyed for their temperature profile during chilled storage and distribution to retailer. The physical and chemical properties and total viable count (TVC) determinations were carried out after the survey. The time and temperature survey that have been conducted in this study could provide knowledge and preventive action to maintain the proper temperature of cold chain for sausages industries in Thailand. It also provides the beneficial proven data on time-temperature history of sausages for manufacturer to predict the pathogen growth in cold chain system.

MATERIAL AND METHODS

Materials

The samples of chicken sausages were produced by processed chicken sausage manufacturer in Pathum Thani, northern of Bangkok. Two surveys were conducted to determine for their temperature profile during distribution to retailer .The temperature survey was conducted in two replicate. Forty packs of sausages amounted 20 kg were determined for their temperature profile during distribution to retailer.

Temperature inside the sausage packages were recorded at an interval of two minutes during distribution. Four unrefrigerated trucks were selected to deliver the sausages in four different routes in Bangkok.

Combase®(Combine Database for Predictive Microbiology) Software was used as a tool to predict the possible pathogens that will grow during distribution period based on worst result in this survey. ComBase is a highly useful tool for food companies to understand safer ways of producing and storing foods. This includes developing new food products and reformulating foods, designing challenge test protocols, producing Food Safety plans, and helping public health organizations develop science-based food policies through quantitative risk assessment. Over 60,000 records have been deposited into ComBase, describing how food environments, such as temperature, pH, and water activity, as well as other factors (e.g. preservatives and atmosphere) affect the growth of bacteria. Each data record shows users how bacteria populations change for a particular combination of environmental factors. Mathematical models (the ComBase Predictor and Food models) were developed on systematically generated data to predict how various organisms grow or survive under various conditions.

Methods

Physico-chemical analysis after distribution

All the physico-chemical analysis were conducted after the sausages were transferred from the industrial chiller and stored in 0°C laboratory chiller for overnight. pH, Aw and acidity were analyzed in triplicate.

Prediction on microbial quality of chicken sausages during storage and distribution

Data on time-temperature survey during chilled storage and distribution were collected and analyzed. The worse case data on those surveys was selected as a representative scenario to predict pathogen growth using an existing mathematical model (ComBase® Predictor 2.0). The ComBase® software was used to predict the response of the microorganisms likely the pathogen such as *Staphylococcus aureus, Salmonella* spp., *Listeria monocytogenes, E. coli, Clostridium perfringens* and *Pseudomonass* spp to environmental factors, such as (pH, aw and acidity), in addition to temperature abuses during storage and distribution of sausages to the retailer. The input parameters for prediction of pathogen growth were initial level load of selected pathogen, maximum temperature, observation period (time) and environmental factors like pH and acidity.

RESULTS AND DISCUSSION

Worst case scenario data chicken sausages during distribution

Data from surveys were taken and analyzed after distribution. Worst case/results from temperature surveys from both survey was selected to predict the potential growth of selected pathogens by using predictive software ComBase. After the distribution pH slightly decreased and the acidity of the sausages were slightly increased. Based on the analysis pH of the sausages was ranged 6.38 to 6.42 and the acidity of the sausages was about 0.06-0.07 (Table 2). The initial acidity of the sausage after cooked was 0.042%. The result shows that the acidity value of the sausages increased after distribution. The results also showed that, there was a relationship between pH and acidity of the sausages after distribution. After the distribution the sausages became more acidic compared to fresh sausages after cooking. It was probably due to lactic acid bacteria fermentation during the delivery period with higher temperature.

No of survey	data recorded	location	Area/zone delivery	Max temp (°C)	Min temp (°C)	Temp differences (°C)
1	20	5	4	23.7	4.2	19.5
2	20	5	4	20.4	7	13.4

Table 1: Summary of temperature survey results chicken sausages during distribution

Survey	pH	Acidity	Aw
1	6.45	0.06	0.98
2	6.38	0.07	0.98

Table 2: Physicochemical analysis of chicken sausages during distribution
*All analysis were done in triplicate

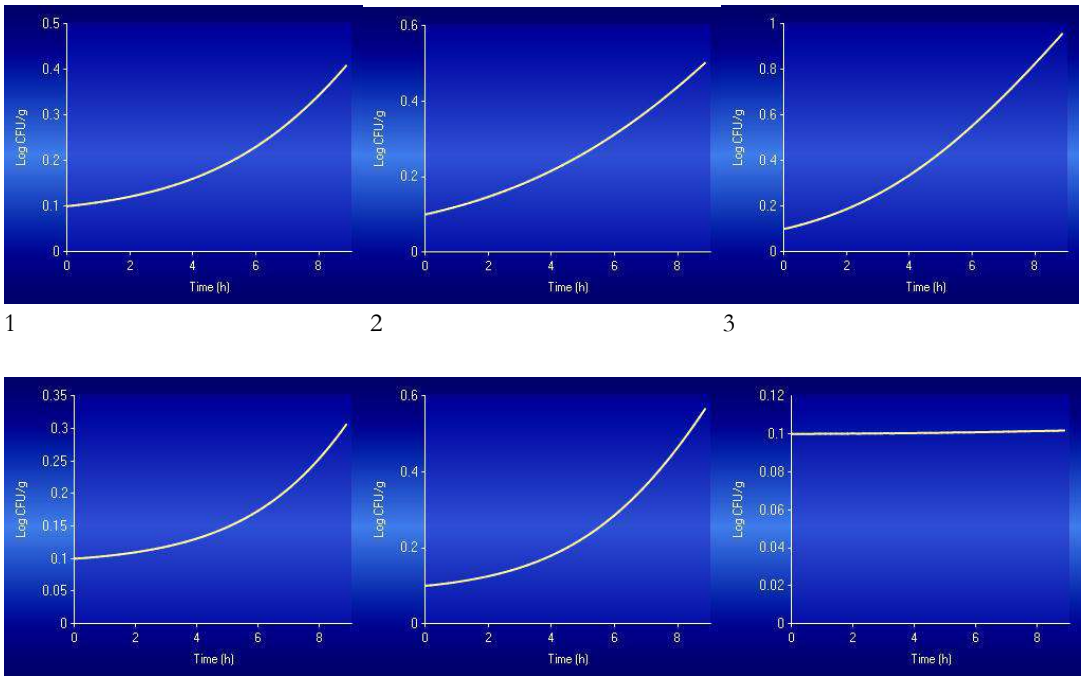
Prediction on microbial quality of chicken sausages during distribution

The worst case temperature data was selected from temperature survey of chicken sausage during distribution to retailer with different delivery time in four different zones. All the parameters and the condition were collected and the predictive analysis was conducted using the ComBase® microbial predictive software to predict the growth of pathogen that could possibly grow during this period. Six pathogens, which were *Pseudomonas* spp., *Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes, Escherichia coli* and *Clostridium perfringens,* were selected for simulation during distribution (Table 3). Five parameters were selected to predict the growth of selected pathogens during chilled distribution as listed in Table 3.

Type of Pathogen	Maximum temperature (°C)	Observation period (hours)	pH	Water activity (a_W)	Initial level of log (cfu/g)	Max rate (log.conc./h)	Double time (hours)
E. coli	19.5	9.05	6.42	0.98	0.1	0.114	2.65
Staphylococcus aureus	19.5	9.05	6.42	0.98	0.1	0.185	1.63
Salmonella spp.	19.5	9.05	6.42	0.98	0.1	0.151	1.99
Listeria monocytogenes	19.5	9.05	6.42	0.98	0.1	0.169	1.78
Pseudomonass spp.	19.5	9.05	6.42	0.98	0.1	0.174	1.73
Clostridium perfringens	19.5	9.05	6.42	0.98	0.1	0.081	3.74

Table 3: Pathogen prediction parameter, rate and doubling time during delivery to retailer

Simulations Pathogens Prediction Six pathogens were simulated using the worst case scenario based on the real data on temperature survey of chicken sausages to retailer around Bangkok area. The result from the prediction showed that *Clostridium perfringens*, *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus*, *E.coli* and *pseudomonas* spp count ranged from 0.11 log cfu/g to 1 log cfu/g during distribution at 19.5°C for 9.05 hours. *Pseudomonas* spp showed the highest growth while *Clostridium perfringens* showed the lowest growth during distribution (Fig 1).All the pathogens was grew rapidly after 4 hours during delivery except for *Salmonella* and *Clostridium Perfringens* after 3 hours and stagnantly while distribution time. Pin et al. (2011) performed growth prediction of *Salmonella* spp. in pork sausages using ComBase® growth model fitted with combining fluctuated temperature condition, pH and Aw in the system. It was reported that the growth rate of *Salmonella* spp decreased gradually when the temperature decreased below 10°C and *Salmonella* population was not seen during storage at 4°C for 8 days of storage (Pin et al., 2011).



1 2 3

4 5 6

Fig.1. : Above from left Growth prediction of *Salmonella spp.* (1) growth prediction of *Escherichia coli (2),* growth prediction of *Pseudomonass* spp(3), growth prediction of *Listeria monocytogene (4)*, growth prediction of *Staphylococcus aureus (5)* and growth prediction of *Clostridium perfringens (6)* during distribution at 19.5°C, pH: 6.42, aw 0.98 and for 9.05 hours observation period

According to Baranyi and Roberts (1994), the ComBase® growth models are able to predict the course of bacterial growth under a temperature profile that changes with time. They also reported that the simplest approach was based on the specific growth rate of the bacterial population in response to temperature changes. Koseki, (2009) developed a new database known as Microbial Responses Viewer (MRV) for 16 types of microorganisms. This MRV was calculated based on temperature, pH and water activity (aw) that was extracted from ComBase® database and the advantage of this database is able to provide the growth or no growth of specific microorganisms (Koseki, 2009).

CONCLUSIONS

Based on the results during distribution, *Clostridium perfringens*, *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus*, *E.coli* and *Pseudomonas* spp count ranged from 0.11 log cfu/g to 1 log cfu/g at 19.5°C for 9.05 hours. *Pseudomonas* spp showed the highest growth of 0.1 to 1 log cfu/g. As a conclusion, higher the temperature and longer the observation period could increase the growth rate of selected pathogens post distribution.

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