TEMPERATURE SURVEY AND MICROBIAL QUALITY OF CHICKEN SAUSAGES DURING STORAGE AND DISTRIBUTION IN COLD CHAIN

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การสำรวจอุณหภูมิและคุณภาพด้านจุลินทรีย์ของไส้กรอกไก่ระหว่างการเก็บรักษาและการกระจายในห่วงโซ่ความเย็น

นายอัสฮาดิ บิน ยาคอบ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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Field of Study	Food Science and Technology
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อัสฮาดิ บิน ยาคอบ : การสำรวจอุณหภูมิและคุณภาพด้านจุลินทรีย์ของไส้กรอกไก่ระหว่างการเก็บรักษาและการกระจายในห่วงโซ่ความเย็น. (TEMPERATURE SURVEY AND MICROBIAL QUALITY OF CHICKEN SAUSAGES DURING STORAGE DISTRIBUTION IN COLD CHAIN) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: รองศาสตราจารย์ ดร. จิรารัตน์ อนันตกูล, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม: ผู้ช่วยศาสตราจารย์ ดร. ชื่นจิต ประกิตชัยวัฒนา, 112 หน้า.

งานวิจัยนี้ได้สำรวจอุณหภูมิของไส้กรอกไก่ในระหว่างการเก็บรักษาโดยการแช่เย็นและการกระจายสินค้าโดยผู้วิจัยได้บันทึกข้อมูลอุณหภูมิและเวลาในระหว่างการเก็บรักษาและการกระจายสินค้าดังกล่าวก่อนจะนำไส้กรอกมาวิเคราะห์สมบัติทางเคมี กายภาพ และจำนวนจุลินทรีย์ทั้งหมดหลังการเก็บรักษา ในการผลิตไส้กรอก ไส้กรอกจะถูกทำให้สุกที่ 80°C เป็นเวลา 5 นาที หลังจากนั้นจะถูกทำให้เย็นจนถึงอุณหภูมิห้องก่อนที่จะนำไปบรรจุใส่ถุงพอลิเอธีลีนขนาด 0.5 กิโลกรัม เมื่อบรรจุเรียบร้อยแล้วจึงนำไปแช่ในอ่างน้ำแข็งผสมน้ำเพื่อลดอุณหภูมิโดยทันทีก่อนที่จะนำไปเก็บในห้องแช่เย็นขนาด 55.2 ลบ.ม. จนกว่าจะขนส่งออกไปจำหน่าย จากผลการสำรวจ พบว่า ในระหว่างการบรรจุ ไส้กรอกมีอุณหภูมิในช่วง 21.2 ถึง 31.5°C เมื่อนำไปแช่ในอ่างน้ำแข็ง ไส้กรอกมีอุณหภูมิลดลงจาก 26.8 ถึง 5°C ภายใน 120 นาที อุณหภูมิเฉลี่ยสูงสุดที่วัดโดยใช้อุปกรณ์บันทึกอุณหภูมิจำนวน 18 จุด (DL1-DL18) ในระหว่างการเก็บแบบแช่เย็น คือ 3.9°C (DL15) ในครั้งที่ 1 ของการสำรวจอุณหภูมิ และพบว่า Total Plate Count (TPC) สูงที่สุด 5.25 log cfu/g ในถุงไส้กรอกที่วางอยู่ในชั้นวางด้านหลังด้านซ้ายของห้องแช่เย็นในครั้งที่ 2 ของการสำรวจอุณหภูมิ การเปลี่ยนแปลงของอุณหภูมิในระหว่างการเก็บมีผลต่อปริมาณจุลินทรีย์ทั้งหมดและค่าแรงเฉือนของไส้กรอก หลังการเก็บไส้กรอกจะถูกกระจายไปยังร้านค้าในเขตปริมณฑลของกรุงเทพมหานครในระหว่างเวลา 19.00 น. ถึง 5.00 น. ของวันถัดไป โดยใช้รถบรรทุกเล็กที่ไม่มีเครื่องทำความเย็น ผลการสำรวจแสดงให้เห็นว่าอุณหภูมิเฉลี่ยของไส้กรอกเพิ่มจาก. 6.5°C ป็น 10.3°C ในระหว่างการขนส่งระยะเวลา 7 ชั่วโมง นอกจากนี้ ยังพบว่าอุณหภูมิเฉลี่ยสูงสุดของไส้กรอกอยู่ที่ 17.2°C ซึ่งมีค่า TPC สูงถึง 7.16 log cfu/g ในถุงไส้กรอกที่วางที่ส่วนล่างของรถที่ขนส่งสินค้าในโซน 3 การวิเคราะห์การทำนายการเจริญของจุลินทรีย์โดยใช้ซอฟแวร์ ComBase® แสดงให้เห็นว่าเชื้อก่อโรคเพิ่มขึ้นจาก 0.1 ล็อก cfu/g เป็น 0.2 log cfu/g ในระหว่างการเก็บในห้องเย็นและเพิ่มจาก 0.1 log cfu/g เป็น 1 log cfu/g ในระหว่างการขนส่งในภาวะแวดล้อมจากกรณีที่เลวร้ายที่สุดจากการสำรวจ

ภาควิชา เทคโนโลยีทางอาหาร ลายมือชื่อนิสิต…………………………………………………

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ASHADI BIN YAACOB: TEMPERATURE SURVEY AND MICROBIAL QUALITY OF CHICKEN SAUSAGES DURING STORAGE DISTRIBUTION IN COLD CHAIN. ADVISOR: ASSOC. PROF. JIRARAT ANUNTAGOOL, Ph.D., CO-ADVISOR: ASST. PROF. CHUENJIT PRAKITCHAIWATTANA, Ph.D., 112 pp

Temperature survey was conducted for chicken sausages during chilled storage and distribution to retailer. Temperature history was recorded while physicochemical and total plate count was evaluated after storage and distribution. Chicken sausages were cooked at 80°C for 5 minutes before they were cooled to room temperature before packaged in a 0.5kg polyethylene bag. The sausages packs were then transferred to an ice slush bath for a rapid cooling before they were stored in a 55.2 m^3 chiller until further distribution. The survey revealed that the mean temperature of packed sausages was from 21.2 to 31.5°C during packing. During ice slush cooling, the sausages were cooled down from the average temperature of 28.3 to 5°C in 120 minutes. The highest mean temperature recorded by data loggers (DL1 to DL18) were 3.9°C (DL15) in batch 1 in chilled storage. The highest TPC (5.25 log cfu/g) was found in the sausage pack located at the middle level of the back shelf on the left side of the chiller in batch 2. It was also found that temperature fluctuation had an effect on TPC and shear force of the sausages. In the distribution, the sausages were distributed using an unrefrigerated container truck from 7 pm to 5 am in the suburb of Bangkok. The result showed the temperature of the sausages increased from 6.5°C to 10.3°C during distribution for 7 hours. The highest mean temperature was 17.2°C and the TPC was 7.16 log cfu/g in the pack located in the lower level in truck container during distribution in zone 3. The predictive analysis showed the pathogens grew from an average of 0.1 log cfu/g to 0.2 log cfu/g during chiller storage and from an average of 0.1 log cfu/g to 1 log cfu/g during transportation in selected parameter from the worst case data from the surveys using the ComBase® predictor software.

Department: Food Technology Student's Signature…………………………….

Field of Study: Food Science and Technology Advisor's Signature…………………………….

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NOMENCLATURE

a^* = Redness

ATP = Agreement on the International Carriage of Perishable Foodstuffs

a_w = Water activity

ACC = Aerobic colony count

b^* = Yellowness

DL = Data logger

EU = European Union

FEHD = Food and Environmental Hygiene Department

FSIS = Food Safety and Inspection Service

FSPT = Food Safety Program Template

HACCP = Hazard Analysis Critical Control Point

IFST = Institute of Food Science and Technology

ISO = International Standard of Organization

L^* = Lightness

LAB= lactic acid bacteria

OECD = Organization for Economic Co-operation and Development

RCBD = Randomized complete block design

TA = Titratable acidity

TISI = Thai Industrial Standard Institute

TPC = Total plate count

UK = United Kingdom

USDA = United States Department of Agriculture

WHO = World Health Organization

CHAPTER I

INTRODUCTION

Cold chain issues continued to be main issues addressed in the food industry for temperature sensitive products (Kuo and Chen, 2010). Perishable foods are prone to growth of both spoilage microorganisms and pathogens due to their enriching nutritional nature. High or improper control of chill temperature can lead to hazards. Most of the outbreaks were caused by improper chilling temperature control in cold chain likely during storage and distribution. 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. The products temperature should then be maintained at -1°C ± 0.5°C during transportation to avoid the growth of bacteria and to extend the shelf life. All products in a container can be maintained within ± 1°C of the set point either in refrigerated or insulated container during distribution to the retailer (James, 1996). 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 plate count 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. Thus, the objective of this study was to investigate time-temperature history of chicken sausages during storage and distribution in cold chain and to determine the pertinent microbiological and physical and chemical properties of the sausages.

CHAPTER II

LITERATURE REVIEW

2.1 Food safety and quality of chilled foods in cold chain

One of the oldest methods to preserve foods is chilling. Chilling involves removal of heat in the system to prolong shelf life of several raw produces and minimally processed foods. This food preservation method is considered safe, reliable, able to maintain fresh and provides convenience despite the limited shelf life. Quality of chilled and cook-chill products is considered safe when their nutritional value, taste, texture and appearance remain wholesome during chilled storage. Ideally, food safety is subject to rules and regulations controls whereas food quality is an issue best left to market (Heap, 2000). In many countries especially in developed countries, cold chain applies to more than half of the food consumed. Increasing demand for long-life fresh products generates new risks. These risks are aggravated by mass consumption and new consumption patterns like fast food, shorter cooking times used in order to maintain organoleptic properties. Raspor (2008) reported that, total chain in chilled foods showed that current maintenance of food safety in food supply chain is easily broken down because of different kinds of barriers. Chilling involves the temperature reducing below the point of ambient temperature but normally not below -1°C. Normally chilled foods temperature is between 0°C to 5°C, but it depends on the standard and regulations of the countries. Most of the European countries comply the chilled foods temperature in the range of 0°C to 4°C (Spain and France), but some countries allow the maximum temperature around 8°C like Finland and Sweden. The temperature difference is based on the ambient weather and temperature condition of the countries.

Institute of Food Science and Technology (IFST) classified chilled foods as semi perishable or perishable products wholesome when they are kept in the temperature range over -1ºC (IFST 2004). Reducing the temperature of the foods does not kill potential microbes but slows down the growth of microbes that cause spoilage in the system. Hence, the chilled foods are considered highly perishable if the temperature control and monitoring system are interrupted. The quality and the safety of chilled foods must be maintained throughout food distribution in cold chain. Ensuring safe food in the system became a responsibility and constant task in developed and developing countries. Martin (2000) reported that safety and the quality of chilled foods required minimal contamination during rapid chilling in processing, low temperature storage, handling, distribution and retail store display until delivery to consumer storage.

In order to maintain safety of chilled foods, the Agreement of the International Carriage of Perishable Foodstuffs (ATP Agreement) had set the maximum limit of temperature during transportation in cold chain. The temperature for chilled meat should not exceed 7°C, 6°C for meat products, 4°C for poultry products and 2°C for fish. All the temperatures were prescribed as a guideline in the food cold chain through storage, distribution until to retailer (Martin, 2000). In consumer's perception, chilled foods are seen as fresh and healthy; ideally meet the growing demand for ready to eat meals. At the same time, they have been rapidly adapted to cater consumers' desire for variety, quality of sensory experience and even indulgence in what they eat. In order to maintain the freshness of chilled foods the interruption in cold chain should be avoided.

Temperature control is crucial in cold chain in maintaining the chill condition of food. Temperature abuse causes spoilage and leads to increase of the pathogenic bacteria growth. It was shown that the minimum temperature for common pathogenic bacteria growth in chilled foods ranged from -0.1°C to 1.2°C for psychotropic and 5.1°C to 10.4°C for mesophilic bacteria (Table 2.1). In most cases, chilled foods are more sensitive in temperature fluctuation than frozen foods.

Table 2.1 Minimum growth temperatures of some bacteria found in foods

Class	Bacteria species	Minimum growth temperature (°C)
Mesophilic	*Salmonella* spp.	5.1°C to 8.7°C
	Staphylococus aureus	9.5°C to 10.4°C (for growth)
		14.3°C (for toxin production)
	Escherichia coli	7.1°C
Psychrotrophic	*Listeria monocytogenes*	-0.1°C to +1.2°C
	Yersinia enterocolitica	-0.9°C to –1.3°C
	Aeromonas hydrophilia	-0.1°C to +1.2°C

Source: Martin (2000)

More precaution steps and awareness have to be taken into consideration to control all stages in chill chain that could reduce the initial microbial load in handling and processing of chilled foods. Temperature control in the cold chain system could also preserve nutritional and sensory attribute of chilled foods.

Furthermore, it is necessary to control the temperature with efficient equipment and appropriate handling in order to control the end quality of chilled foods. According to data reported by WHO, in Slovenia the most common infection involved in food poisoning was caused by *Salmonella Enteridis* from eggs and poultry products (IVZ, 2006). Rangel et al. (2005) reported that about 21% of food borne outbreak in United States was caused by *Escherichia coli* 0157:H7 that was related to consumption of chilled foods. It has been shown some of the outbreaks in United States and Slovenia were caused by temperature abuse or incorrect refrigerated temperature in cold chain.

2.2 Controlling the good system in food cold chain

Strict temperature control in cold chain could minimize the risks of potential pathogenic bacteria. Lowering the temperature of fresh produce especially for leafy vegetables is easy to retain the most favorable product in optimum quality that could decrease the physiological process such as transpiration, respiration and the chemical changes in fresh produce (Rediers et al., 2009). Undesirable storage temperature may cause losses of color pigment, discoloration, tissue softening and undesirable ripening stage in the products (Brosnan and Sun, 2001). 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).

HACCP plans need to be set up in order to meet general food safety requirements in the cold chain started from processing until the retail level. The public authorities or the government agency should play an effective role to control the system to ensure consumer protection. In consider that, the retail outlets should be audited by an independent organization that will prove to the food retailer, which they demonstrate a good level of temperature control. It shows a favorable assumption when they were inspected. Then, a further step that has to be taken is by determining the framework of reference to the audit. The frame of reference is designed to enable retailers to assess their own situation (Janez, 2008). It comprises a good collection of specifications that can be used when retail outlets are audited by independent organizations.

The frame of reference was compiled bear in mind by ISO 9001: 2000 Standard and HACCP specification. The procedure allows for expansion and for those who want to improve their equipment. The first section of the frame of reference concerns the operation and organization of refrigerating plants. It was based on general inspection, temperature recordings and attempts to identify reasons for temperature variations by analyzing the way refrigerating plants are designed, used and maintained. Audits undertaken by independent organization could improve the cold chain system in retail food (Janez, 2008). The auditing process could make the food company to identify the areas that need to be improved and maintained like the temperature control during storage and distribution with comply the existing standards such as EN 441 for the selection of refrigerated equipments (Janez, 2008).

2.3 Quality of sausages and meat products

Cooked 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).

Billiard (2003) suggested that, if the interruption of cold chain occurs, the consequences may take various forms of incident like development of spoilage microorganisms that may cause food borne illnesses. 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. In conclusion, food safety and quality of sausage and meat products must be maintained throughout all the processes until the food is distributed.

2.4 Temperature survey of refrigerator temperature in different countries

Refrigerators are the most common cooling appliances used for storage and preservation of foodstuff. Billiard (2005) reported that, there was close to one billion household fridges worldwide. The prime use of domestic refrigeration systems is for food storage and preservation. There were 1.7 refrigerators per household in France and approximately 30% of domestic refrigerators production rising steadily in developing countries in the year 2000 (Billiard, 2005). Refrigerator is one of the main energy consuming electrical equipment in the household. Razali and Othman (1993) unveiled that 76% of total houses in Malaysia were equipped with at least one refrigerator. Refrigerator or fridge is a compulsory home appliance and very few households in some developing countries did not own a fridge for storage of chilled foods (James, Evans, and James, C., 2008). In developed countries like in United

Kingdom, the market penetration for domestic refrigerator is more than 99% that reported by AMA Research (2003). Nowadays, the consumers are more demanding to partially cooked and ready to eat foods (RTE foods).

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, Banwet, and Shankar, (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 (2003) 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.

Monitoring the temperature is crucial in the cold chain management system. The chill chain network involves in all operation system beginning with raw material preparation until delivery to the consumer's storage. Most of the temperature surveys in domestic refrigerators showed that, majority of the consumers did not aware of and care about their refrigerator temperature (James, 2003). Joshi et al. (2010) reported that 72.9% of respondents in three states of India did not know their domestic temperature refrigerator. James (2003) also reported that, in France, about 80% of the fridge's temperatures was above 5°C and in Greece, 50% of home refrigerators was above 9°C. In Irish surveyed from 1020 respondents throughout the island of Ireland, there was about 22.4% of respondents aware of the right refrigeration temperature that it should be in the range of 1°C to 5°C, while 68.6% did not (Bolton, Kennedy, and Cowan, 2005). There was only 23.2% of surveyed domestic refrigerators had a thermometer (James, 2003). In a survey of refrigeration temperature profile over a period of 72 hours conducted by Bolton et al. (2005) 40 out of 100 fridges that have been surveyed had the control temperature in the range of recommended temperature (0°C to 5°C), 54 had the temperature in between 5°C and 10°C and the rest six fridges had an average temperature higher than 10°C.

Louise and Thomson (2009) reported that an average sample size for surveys should be at least 6 per 1000. However in UK it is suggested that the sample size should be about 1 per 1000 population. It means that 100 samples should be taken in the 100000 population. There are two kinds of domestic refrigerators that are available in the market nowadays, a static type and ventilated system. The common is static type that is widely used at home. This refrigerator type used the principal of heat transfer by natural convection and airflow is due to variations in air density (Laguerre and Flick, 2004). Laguerre and Flick (2004) also reported that, several consumers occasionally stored their perishable foods in fridges running at high temperatures for a long time. A survey in France showed that there was 26% of refrigerators running at an average temperature over 8°C whereas the recommended temperature was 4°C (Laguerre, Derens, and Palagos, 2002). In France survey, the temperature varied within a range of 7°C to -12°C with the average value of -1.2°C.

In a Dutch study cited by Notermans et al. (1997). 70.4% of refrigerators temperature was above 5°C and only 3.2% of those was at or over 9°C. James et al.(2008) reported that, in a USA survey of product temperatures from 939 refrigerators, there was only 235 units (27%) where the temperature was over 5°C after transportation of meats products and cheese to homes and storage for 24 hours and the lowest product temperature recorded after 24 hours was -6°C and the highest was 21°C. Sergedilis et al. (1997) reported that, 75 units of domestic fridge samples in Greece had temperatures equal or above 9°C and 25 units (13.6%) had temperatures exceeding 10°C. James et al.(2008) reported that, in a study by Laguerre et al.(2002) in France the temperatures were recorded at the upper, middle and bottom of 119 refrigerators at 2 to 8 minutes interval over 7 days using a data logger. The result showed that more than 50% of temperature was above the 5°C and only 12% the temperature was below 5 °C.

In a UK study by Evans (1992), the temperatures profile in each refrigerator showed that the mean temperature recorded from upper, middle and bottom sensors over 7 days ranged from -1°C to 11°C. The overall mean of ambient temperatures of the refrigerators in this survey was 6°C with 70% of refrigerators operating at mean temperatures greater than 5°C. In the UK survey by Evans et al. (1991), three air and two product sensors were placed in the refrigerator to monitor temperatures at eight seconds interval, the average mean temperatures was about 7°C. Most of the temperature surveys of household refrigerators used calibrated thermometer or portable mini temperature recorder like data logger to ensure the correct temperature in air temperature measurement in different parts of refrigerator. In most cases, the popular sites of temperature monitored were at different part of shelves (top, middle and bottom) in refrigerators and at the door of the fridge.

FAO suggested that the temperature of refrigerators has to be monitored regularly by using thermometers to assure the correct temperature that is recommended. This thermometer should be placed in an easy-to-read location and the temperatures should be checked regularly for at least once a week (Laguerre et al., 2002). It was shown that, different types of food in consumer refrigerators required different temperatures range of storage as listed in Table 2.2 (Laguerre et al., 2002).

Table 2.2 Preservation temperature of some food products to be maintained until consumption

Temperature	Foodstuffs
0–2°C	Fish and seafood
2°C	Ground meat
4°C	Various types of meat and meat preparations sausage, chicken, fresh milk, cheese
5 °C	Eggs
8 °C	Stable meat products, dairy products

Source: Laguerre et al., (2002)

The air temperature did not give an effect on the refrigerator temperature and there was no correlation between the thermostat setting with refrigerator temperature (Laguerre et al., 2002). It was found that certain refrigerators, in spite of setting at the coolest position, the temperature is still higher than 5°C. Temperature measured using a thermometer did not represent the corrected operating conditions of the refrigerator. In UK, there was 32.8% of consumers setting their refrigerators according to the weather condition, for example, setting the refrigerator to a lower temperature in the summer season (James et al., 2008). Sometimes the temperature in refrigerator was higher than recommended temperature but there was no clear relationship with food poisoning.

2.5 Incident of food pathogens contamination in chilled foods

Inadequate cooling of refrigerators was always cited as one of a feasible cause in food poisoning incidents in recent days. Food safety is a vital component of consumer’s health issues and concerning food industries in developed and most of developing countries all over the world. The incident and the outbreak of food borne diseases rose in developing countries, as well as in the developed countries. These issues become a major problem in some of undeveloped countries. Laguerre and Flick (2004) reported that, most of the incidents was due to temperature abuse in the chill chain. Temperature abuse can accelerate microbial growth in foods to some extent. The population of certain bacteria could double if temperature rises 5°C for only just 10 minutes in the optimum bacteria condition. *Listeria* contamination was increased from a few CFU per gram to more than thousand CFU per gram at 10°C for less than eight day of storage (Laguerre and Flick, 2004). It was reported by several researches that most of the food poisoning cases came from unhygienic domestic refrigerator environment that was associated with incorrect food handling and improper hygiene attitude of consumer (Evans, 1992). In consumer's survey by Evans-(1992), the consumers believed that the shelf life of fresh and refrigerated foods in a home refrigerators is two days or shorter. However, in the real situation most of the consumers stored their fresh food more than the time that have been suggested.

Joshi et al. (2010) reported that, the distinct possible factors causing the illnesses included the arrangement of fresh meat, poultry or seafood at the upper shelf of a refrigerator without cover or properly packed. Arrangement and placement of foods in a fridge could influence cross contamination among the foods in the shelves and affected the shelf life of the other foods in the refrigerator. About 38% of food stored in home fridge involved a potential risk of cross contamination from juices that were leaked and dripped on other foods in the refrigerator. These findings emphasize the need for greater consumer education regarding appropriate refrigerator cleaning and safe food handling practices to prevent food borne diseases incidents. Jevsnik, Hlebec, and Raspor, (2008) reported that, the proportion of respondents who washed their hands properly before preparing foods was 86%. This number included the person who only washed their hands based on what they were doing previously (7.9%) and what type of food they were going to serve (5.8%).

There was about 66% of the consumers who did not wash their hands prior to work, 41% did not wash vegetables, and about 60% used the same cutting board for all cutting tasks. Jevsnik et al. (2008) reported that the number of consumers that washed their hands appropriately was (45 %) lower than was recorded from other similar studies . A better results from similar studies were attained from consumers of Ireland (64.6%) from study by Kennedy et al. (2005), Trinidad (78.3%) in Badrie et al. (2006) and Australia (82.3%) in Jay, Comar and Govenlock *(*1999). Kennedy et al. (2005) reported that bacteria contaminated from unwashed raw foods, leaking packages, hands, surfaces, that was introduced to domestic refrigerators might directly contaminate other stored foods. The internal surface of the refrigerator poses risks of indirect longer term contamination during subsequent food preparation activities. Higher total viable counts and total coliform count in many domestic refrigerators indicates the potential of these domestic appliances as important sources of food contamination during domestic food storage (Kennedy et al., 2005). A proper and the best way for hand washing before handling of food preparation is by using lukewarm water with soap or special detergent and scrubbing action on both hands at least for 20 seconds (Jay et al.,1999).

Patil et al. (2004) summarized that the number of food borne diseases increased, clearly indicating poor consumer practices related to intake of fresh or uncooked foods which was not washed properly. Furthermore, poor hygienic practices were also the main factors causing outbreaks of food borne illnesses. In Slovenia, the survey was conducted to determine the level of consumers handling of foods prior to storing in a domestic fridge. The set of questionnaires were based according to gender, age, marital status, education level, food safety knowledge, food safety practices and food handling practices in home. Almost 40% of 1030 respondents did not notice the temperature of their own refrigerator. More than half (53.5%) of the respondents let the leftovers of the foods cool at room temperature before putting them back in their fridge and 12.5% left leftovers on the kitchen until they were eaten (Jevsnik et al., 2008). In best food handling practices the leftovers of the foods should be kept in a clean containers or wrapped and covered , cooled as fast as possible and put in the fridge at 4°C to 7°C for no more than three days of storage. If these foods are stored in chilled condition more than two days, the possibilities of pathogens growth are high and can cause incidents of food poisoning (Jevsnik et al., 2008).

Personal hygiene practices should be regularly practiced by consumers at home before preparing food and before preserving food items in the fridge. Basic hand washing technique should be applied in order to maintain the cleanliness of hand while handling the foods before storage and cooked. Proper package and cover should be applied to perishable items before storing in a refrigerator to avoid cross contamination and dripping from other foods in the refrigerator. James et al. (2008) reported that numerous data from published surveys indicated that most of refrigerators around the world were running beyond the recommended temperatures. James et al. (2008) also reported that fewer cases of *Listeria* contamination in Portugal was found; there was only 3.5% from 86 units of refrigerators that were surveyed. Azevedo et al. (2005) reported that, even though *Listeria* spp. was found in fridges but it did not seem to correlate with the frequency of cleaning and the types of cleaning agent appeared to be important factors. Sergedilis et al. (1997) reported that *Listeria* spp. had the capacity to attach to utensil and tool surfaces of food producing biofilm that resists cleaning and sanitizing. The plastic surface interiors of household fridge was suggested to be cleaned using chemical agents like bicarbonate solutions for cleaning purposes to restrain the growth of yeast and molds. The alkaline solution could also minimize bacteria growth.

Improper cleaning and incorrect choosing the right sanitizing agent will not eliminate or reduce the pathogen in household refrigerator. Following the appropriate cleaning procedure with the right solution could prevent the growth of some bacteria in refrigerators. Referring to European Commission (EC) regulation 2073/2005, RTE foods should not surpass the limit of *Listeria* spp of 2 log of cfu/g for *Listeria* spp throughout their shelf life. This regulation was applied to RTE foods both able and unable to sustain the growth of *Listeria monocytogenes* and this last group includes food products with pH value less or equal 5 and the water activity (a_w) less or equal 0.94 with the shelf life shorter than five days.

It is strongly believed that it was important to tell the consumers and manufacturers about the importance of the right storage temperature of RTE foods. The consumers required more information about the importance of food safety handling practices and appropriate refrigeration temperature. Garrido et al. (2010) reported that, in several studies about safe refrigeration temperature, there was about 87.9% from 1975 respondents who were interviewed did not aware of their fridge temperature. It was necessary to improve consumer education regarding food safety in storage practices in chilled foods.

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

3.1.1 Chicken sausages

- The sausages, comprising 72-75% chicken, 15-19% water, 3-5% modified starch, 1-2 % sugar, and 0.5-1.0% salt were collected from a meat processor in Pathum Thani province located in Sub-urban area north of Bangkok.

3.1.2 Chemicals and apparatus

The following chemicals were used in this study:-

-Sodium hydroxide (LOBA Chemie Pvt. Ltd. Mumbai , India)

-Filter paper (Whatman^{TM} no. 1 diameter 110mm)

-Peptone from meat (Merck KGaA, Darmstadt, Germany)

-Plate count Agar (HiMedia Laboratories Pvt. Ltd. Mumbai, India)

-Disposable petri plate (Hycon plastic ,PS Petri dish)

3.2 Instruments

-pH meter (pH 700 Eutech Instruments Thermo scientific, USA)

-Blender (Philips 400W HR2021, Holland)

-Texturometer model TA-XT2, (Stable Micro Systems, Godalming, UK)

- Chromameter (CR400 series Minolta Tokyo, Japan)

-Incubator (Memmert, GmbH+Co.KG, Nuremberg Germany)

-Autoclave (Tomy SX-700, Tokyo, Japan)

- Vortex (Scientific Industries, USA)

-Electronic balance (Mettler Toledo, Switzerland)

-Stomacher 400 circulator (Seward Stomacher, England)

3.3 Methods

3.3.1 Temperature survey of chicken sausages: post process handling and storage

The temperature survey of process handling and storage of chicken sausages was conducted 6 times over a period of 5 months. In each survey, nine kilograms (18 packs) of sausages, accounting for 10% of each production batch, were monitored for their temperature experience. All samples were later analysed for microbial quality and physico-chemical quality.

Figure 3.1 shows some processing stages and data logger placement

Cooked sausages on metal racks

Cool in an ice slush bath

Remove the sausage casing

↓

Pack in 18 bags with mini

temperature data logger

↓

Sausage packs with data

logger placed in the center

of the pack

↓

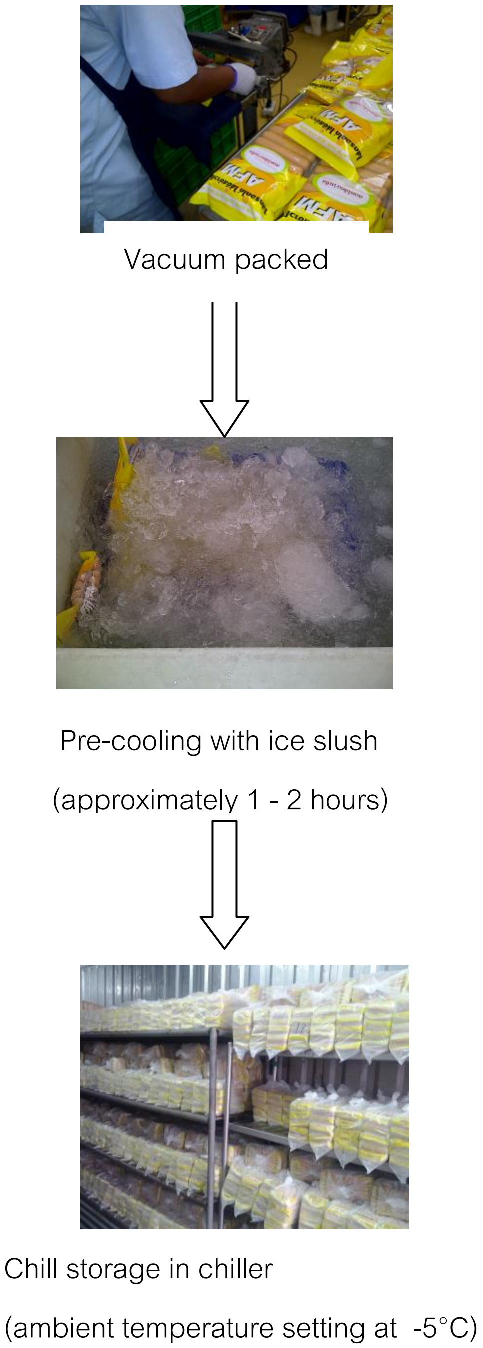


Figure 3.1 Flow diagram for post process handling of chicken sausages until chill storage

There were four types of sausages that was analyzed on this survey. The types of sausages were as followed:-

1. Survey number 1: corn cheese
2. Survey number 2: smoky bite

2 days of chilled storage

3. Survey number 3: corn cheese
4. Survey number 4: squid sausage
5. Survey number 5: corn cheese
6. Survey number 6: hot dog

7 days of chilled storage

The industrial chiller temperature was set up around -5°C to ensure the temperature of the sausages in the range of 0° to 2°C. The mini temperature data logger (RC-1+ mini data logger, Protronics Intertrade Co., Ltd., Bangkok) was used in this survey. The temperature of the sausage packages in the chiller was recorded for two days and seven days in industrial chiller at an interval of two minutes. The chiller dimension was about 55.2 m^3 (2 meter Width × 12 meter Length × 2.3 meter Height). The 18 packs of sausages were placed at 18 different locations (Figure 3.2) in the chiller. The ambient temperature in the chiller was also recorded.

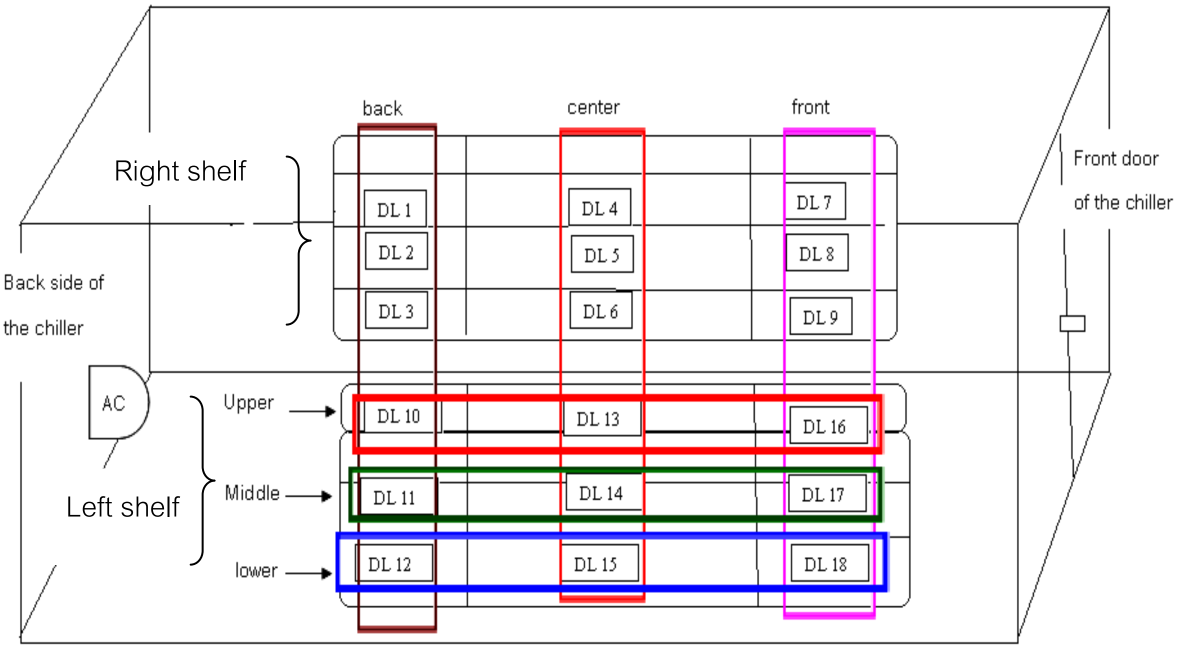


Figure 3.2 Schematic diagram showing data logger locations in industrial chiller at 18 different locations

All the data loggers were sanitized using ninety percent of ethanol spray before they were packed along with sausages in a polyethylene bag. After that, the sausages were vacuum packed before sent to cooling process. The packed sausages were transferred to a tank filled with ice slush (1:1 ice: water ratio) for cooling process about an hour up to two hours depending on the type of sausages. Next, the chilled packed sausages were stored in the specific chiller at eighteen locations (Figure 3.2). The ambient temperature of the chiller, at front and back shelf was also recorded during storage using the data loggers. All the sausages were stored for two and seven days. After that, the sausages were brought back to the laboratory for analyses. The physico-chemical and microbiological analyses were carried out. The temperature history during storage was downloaded via a receiver reader interface through a computer. The temperature profile data during storage were analyzed using Microsoft excel.

3.3.1.2 Microbiology analysis after chill storage

Eighteen packs of the sausages in each survey were taken back from the factory in iced polystyrene boxes to the microbiology laboratory about 1 to 2 hour journey time to determine the total plate count (TPC). All the samples were stored at 0°C overnight in a refrigerator prior to analysis. TPC was determined following the method in Bacteriological Analytical Manual (BAM, 2001; Lynne, 2005). Twenty five grams of chicken sausage sample was aseptically weighed and transferred to a sterile stomacher bag in which it was added with 225 ml of 0.1% sterile peptone water and macerated for 30s at 230 rpm using stomacher paddle (Seward Stomacher model 400, England). Nine milliliters of 0.1% sterile peptone water (Merck, Germany) was used as diluent for making serial dilutions (10^{-2} to 10^{-5}). Then, 1 ml of each diluent was pipetted into the sterile petri plate (Hycon disposable petri plate) and the warm plate count agar (PCA, HiMedia Laboratories Pvt. Ltd. India) was poured into the plate and let stand to solidify. All the plates were incubated in an incubator (Memmert GmbH+Co.KG, Nuremberg Germany) at 35°C± 2 for 48 hours. Plates showing 25–250 colonies were counted.

3.3.2 Temperature survey during distribution to retailers

3.3.2.1 Time-temperature profile during distribution

Time temperature survey for distribution of chicken sausages (squid chicken sausage variant) was taken place around Bangkok area. The survey was conducted in four routes with two batches. Twenty packs of sausages were followed for their temperature history during the distribution in each batch. Twenty packs of sausages amounted 10 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 to the retailer. Four unrefrigerated trucks were selected to deliver the sausages in four different routes in the vicinity Bangkok. The distribution started at 7.10 pm in the evening and finished 7.00 am on the next morning.

Five packs of sausages were placed in five different locations on each truck (Figure 3.3 and Figure 3.4). Arrival, loading, and departure time of the trucks were recorded during the survey. The dimension of the pick up truck container was about 5.148 m^3 (Length: 2.2meter x Width: 1.5meter x Height: 1.56meter) (Figure 3.3).

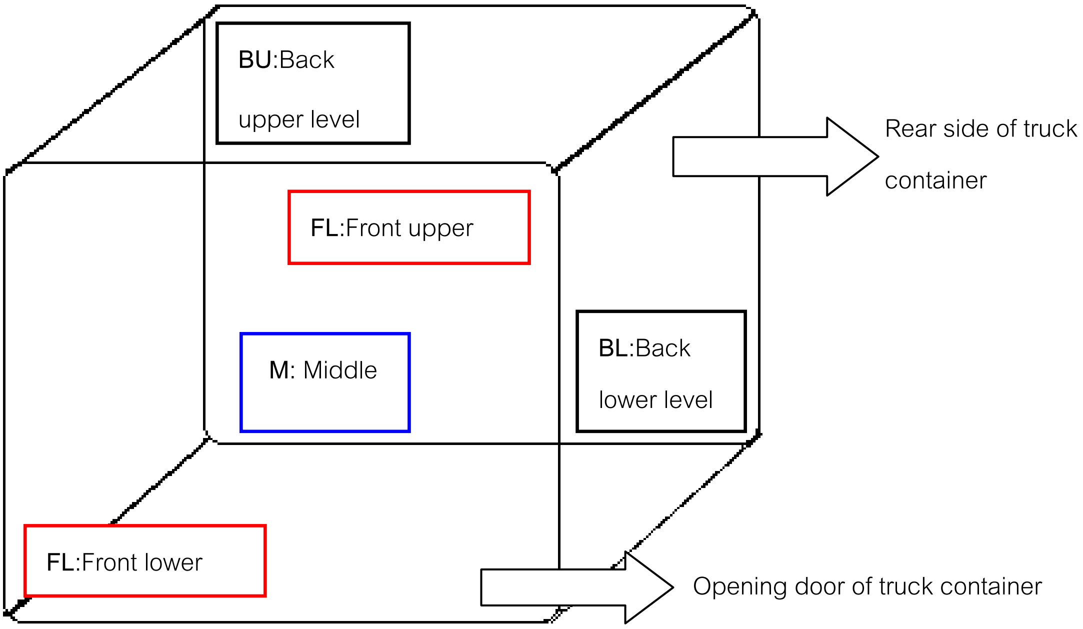


Figure 3.3 Schematic drawing of sausages locations in each unrefrigerated truck container during distribution.

Location of sausages in each truck container:-

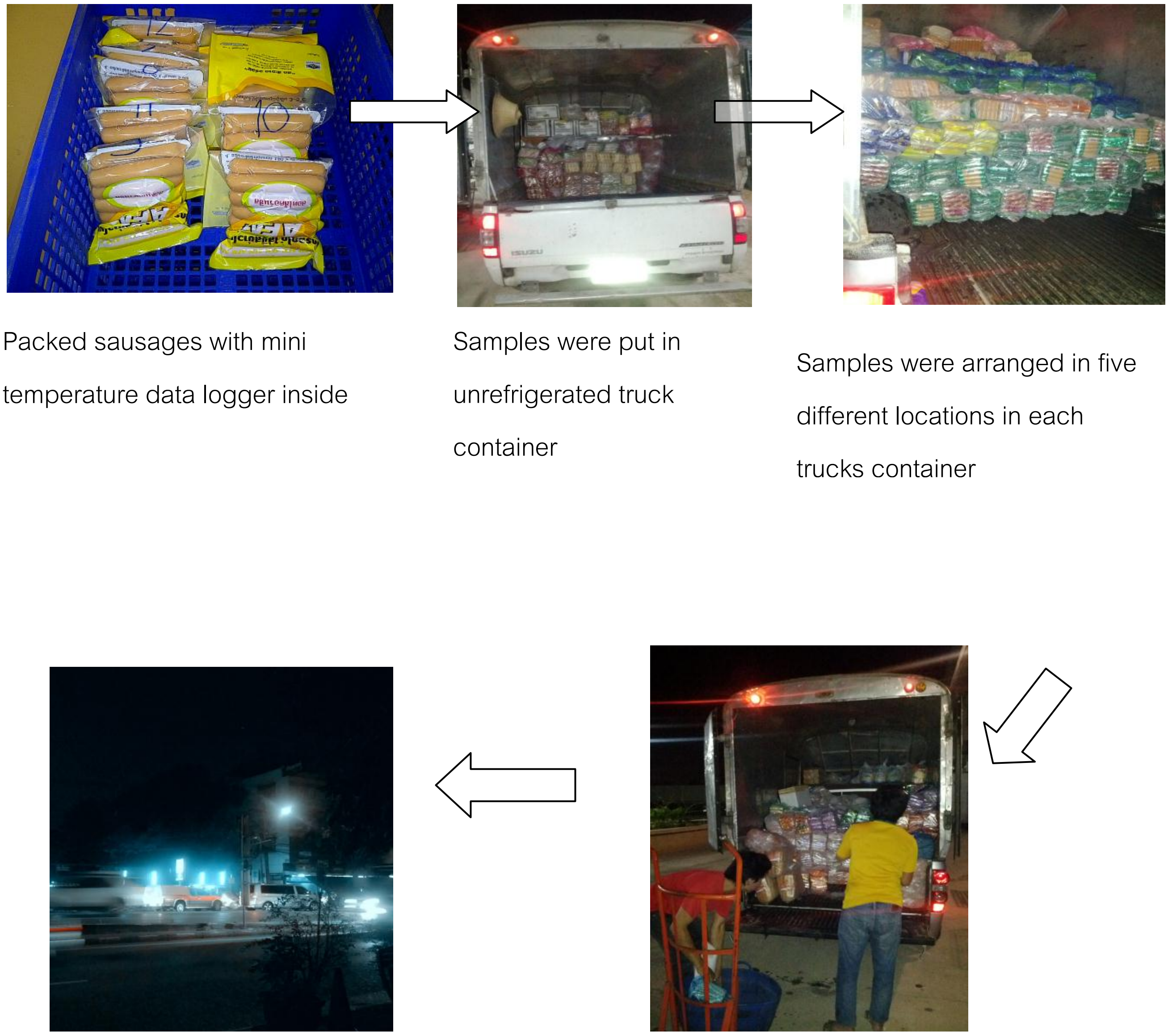
M: center in the middle in truck container

FL: front lower level on the left side of truck container

BL: back lower level on the right side of the truck container

FU: front upper level on the right side of the truck container

BU: back upper level on the left side of truck container



Packed sausages with mini temperature data logger inside

Samples were put in unrefrigerated truck container

Samples were arranged in five different locations in each trucks container

Delivered in Bangkok area

Completed loading the sausages

Figure 3.4 Flow chart of the temperature survey of chicken sausages during delivery (distribution).

3.3.3.2 Microbiological analysis of chicken sausages after distribution

Twenty packed of the sausages placed in polystyrene boxes with ice in each survey were taken back from the factory and transferred to the microbiology laboratory at Chulalongkorn University to determine the total plate count (TPC). All the samples were stored overnight at 0°C in a refrigerator prior to analysis. TPC was determined following the method in Bacteriological Analytical Manual (BAM, 2001; Lynne, 2005) similar to the procedure in 3.3.1.2.

3.3.3.3 Physico-chemical analysis after chill storage and 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.

3.3.3.3.1 Texture analysis

The shear force of the chicken sausage samples were determined by using Texturometer model TA-XT2, (Stable Micro Systems, Godalming, UK) using a Warner Bratzler blade (HDP/BSW). The uniform sized samples (1 cm^3) were radially sheared with a V-shaped blade attached to a plunger at 50 mm/min crosshead speed. The load cell of the texturometer was 30 kg. The mean of ten readings of the data were taken for further analysis (Thomas et al., 2008).

3.3.3.3.2 Color (color space L*, a*, b*)

The color evaluation was analyzed using Chromameter (CR400 series Minolta Tokyo, Japan). Surface color of chicken sausages were determined by color space L*= lightness, a*= redness and b*= yellowness. The chromameter was standardized with a white calibration plate (Y=93.8; x = 0.3158; y = 0.3323) before the measurement. An average value from five random locations of sample surface was used for further analysis.

3.3.3.3.3 pH

Ten grams of chicken sausages was homogenized with 90 ml of distilled water. The pH of all the chicken sausage samples was measured with a glass electrode using a digital pH meter (Eutech pH 700 Thermo scientific, USA). The pH meter was calibrated with standard buffers of pH 4.0 and 7.0 before analyses.

3.3.3.3.4. Titratable acidity

Ten gram of chicken sausage sample was macerated with 90ml of distilled water using commercial blender (Philips 400W HR2021, Holland). The homogenate of the samples was filtered through Whatman no. 1 filter paper. 25 ml of filtrate was titrated using 0.1M sodium hydroxide (0.1N NaOH). Titratable acidity was expressed as ml of 0.1 M NaOH/g sample required to neutralize the filtrate (Wang, 2000; Thomas et al., 2008). The titration was carred out until the pH reached 8.1-8.2.

To calculate the titratable acidity,

$$\% \text{ acid (wt/wt)} = \frac{N \times V \times Eqwt}{W \times 1000} \times 100$$

where:

N = normality of titrant, usually NaOH (mEq/ml)

V = volume of titrant (ml)

Eq.wt. = equivalent weight of predominant acid (mg/mEq)

(Lactic acid, Eq.wt.= 90)

W = mass of sample (g)

1000 = factor relating mg to grams (mg/g)

(1/10 = 100/1000)

Source: simplified from Nielsen (2010).

3.3.3 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, a_w 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.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Temperature survey of chicken sausages during packaging and chilled storage

4.11 Time-temperature profile of chicken sausages during packaging and chilled storage

According to the package label, the shelf life of the sausages in this survey was set for two weeks when stored at 0°C to 5°C. Real time-temperature history of the sample sausages was monitored for two days (survey number 1 and survey number 2) and seven days (survey number 3 to survey number 6) of storage in 18 locations on shelves of industrial chiller in six surveys with two minutes time interval. The 108 data of temperature profiles were analyzed to see the correlation between the mean temperature inside the sausage packages during post-processing storage for two days and seven days and the physicochemical and microbiology qualities. The survey of product temperature was divided into three parts: in the packaging, pre-cooling in an ice slush and storage in a chiller (Figure 4.1 to 4.4).

From the survey, the packing room temperature was around 27° to 28°C, which was much higher than the recommendation of Food Safety and Inspection Service (FSIS), United States Department of Agriculture (USDA) for temperature control of <50°F (10°C) in packing room of products to be refrigerated or frozen. After packing, the temperature suddenly decreased in the ice slush tank during pre-cooling process. The packing of the sausages was taken about 30 minutes and the cooling process was about 1 to 1.5 hour. The results showed that the temperature in packaging room was high and mean temperatures ranged from 21.2 °C to 31.5 °C (Table 4.2 to 4.7).

The reasons why the temperature in packaging was high were as followed:-

- The setting air conditioner in the room was set up high around 22°C and was not enough to cool down the room to appropriate temperature condition.
- Warm air from cooking area (hot area) went to packaging room due to the in out of hot products in the room.
- Hot products coming in the packaging room rises the ambient temperature in the packaging room.

During cooling in ice slush tank, it took almost an hour to reduce the temperature from ambient temperature in packing room to drop below 3°C. The time for cooling process should not be more than 90 minutes to bring down the temperature below 3 °C (FSPT, 2010). It is shown that the time to bring down the temperature below 3°C was about an hour (60 minutes) up to 1.5 hour (90 minutes). Wang and Luo (2011) reported that the finished products must be loaded to a chiller within 30 minutes and the chilled products should be kept between 0°C to 4°C. Salvadori and Mascheroni (2005) suggested that refrigerator temperature should be below 5°C to control the growth of food poisoning microorganisms. Since the packed products were in direct contact with ice slush, the microbial load of the ice slush should also be kept low to avoid hazards due to possible contamination. The total plate count (TPC) of the ice slush used in the cooling process ranged from 2.7 to 3.5 log cfu/g (Table 4.1).

According to risk assessment report of Food and Environmental Hygiene Department (FEHD) in Hong Kong, it was reported that the aerobic colony count (ACC) of ice used in food should not exceed 500 cfu/ml for packed ice and lower than 1000 cfu/ml for loose ice (FEHD, 2005). Other study reported that mean numbers of microorganisms of ice used to cool packed poultry was 3.5 log cfu/ml (Northcutt and

Smith, 2010). It was shown that ice used in cooling process could be source or vehicle for transmission of some pathogenic bacteria in foods.

Table 4.1 Mean total plate count (TPC) of ice cubes used in cooling process of sausages

Sample Ice	Mean total plate count (TPC) log cfu/ml
Large cylindrical ice cube	2.70
Small cylindrical ice cube	3.54

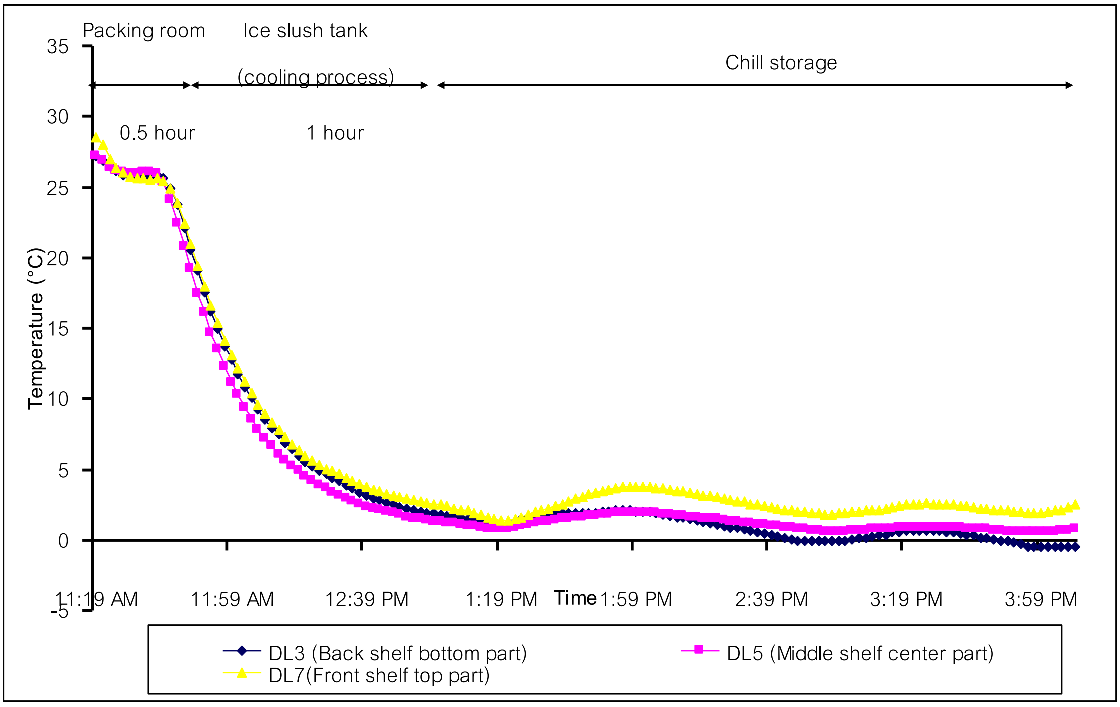


Figure 4.1 Selected temperature profiles of chicken sausages from packing until chill storage. The data was taken from three different locations in chiller (survey 1)

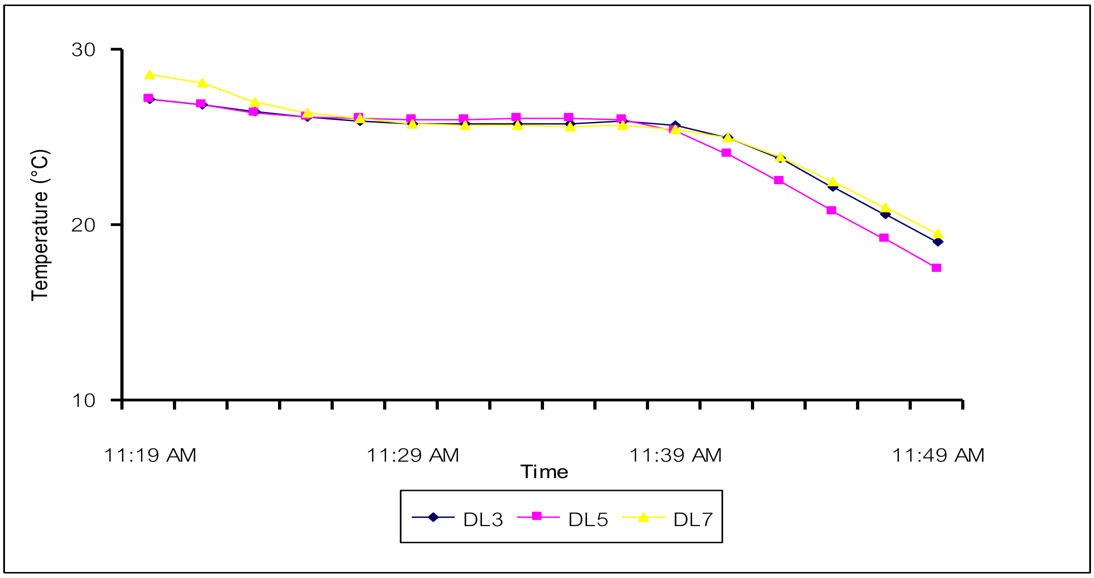


Figure 4.2 Example temperature profile of chicken sausages in packing room from survey 1

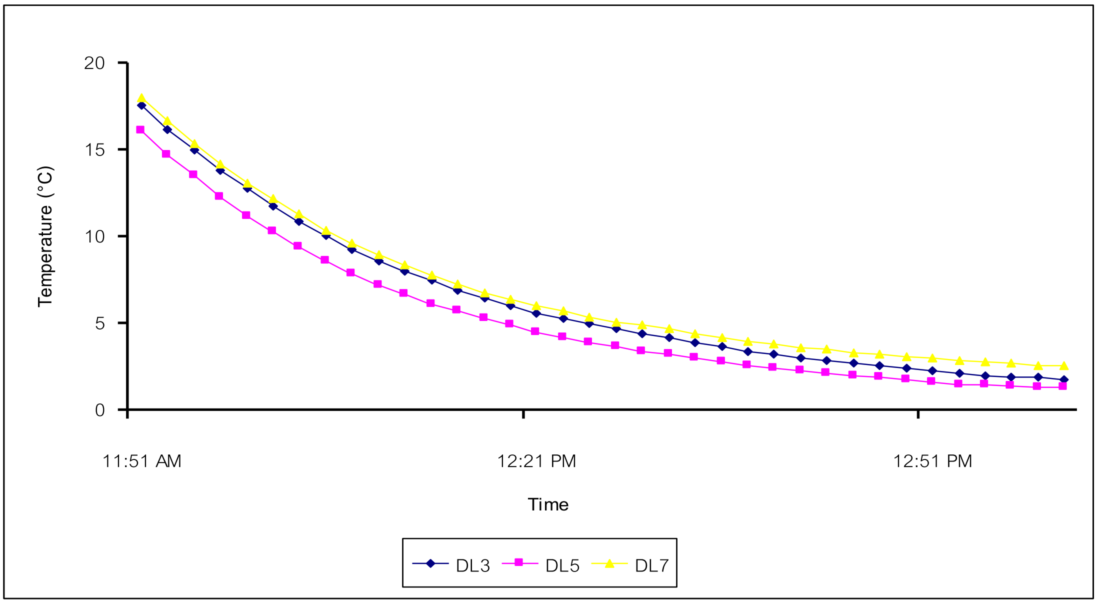


Figure 4.3 Example temperature profile of chicken sausages in ice slush tank (cooling process) from survey 1

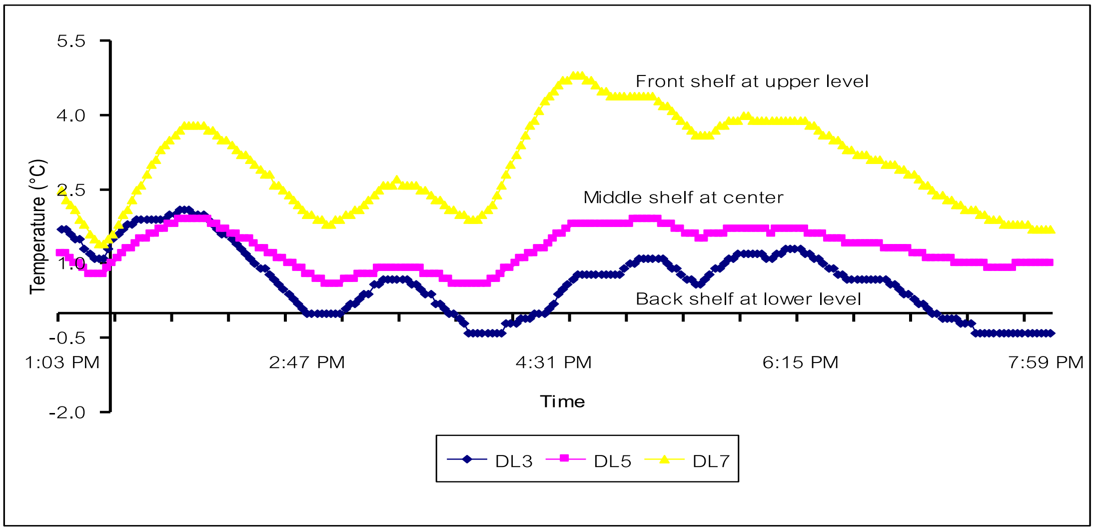


Figure 4.4 Example temperature profiles of chicken sausages during chill storage. The data was taken from three different locations in the chiller during working day (survey 1).

The results of temperature distribution in the chiller showed that the highest mean, maximum and minimum temperatures in survey number 1 were located in the middle shelf lower level on the left side shelf of the chiller (DL15), (Table 4.2). The lowest mean temperature was located in the back shelf at lower level on the right side of the chiller (DL3) as listed in Table 4.2. However, the lowest minimum temperature was located at the back upper level shelf on left side of the chiller (DL10). While the lowest maximum temperature was located at the middle shelf center level and lower level of the right side shelf in the chiller (DL5 and DL6) (Table 4.2). The result showed the highest mean temperature in survey number 2 was located in back shelf upper level on the left side (DL10) of the chiller. The lowest mean temperature located in the front shelf middle level on the left side (DL17) of the chiller. The highest maximum temperature located at the back shelf upper level left side (DL10) and the lowest in middle shelf center level on the right side (DL8) of the chiller. The highest minimum temperatures were located in front shelf lower level (DL9) and back shelf upper level of the chiller (DL10) on the right side of the chiller. The lowest minimum temperature was found to locate in middle shelf lower level (DL15) on the left side of the chiller (Table 4.3).

Table 4.2 Temperature survey result for corn cheese chicken sausage survey umber 1 (2 days of storage)

No. of data logger	Packaging Room			Ice slush tank (cooling process)			Chill storage		
	Max temperature (°C)	Min temperature (°C)	Mean temperature (°C)	Max temperature (°C)	Min temperature (°C)	Mean temperature (°C)	Max temperature (°C)	Min temperature (°C)	Mean temperature (°C)
1	27.6	**19.9**	25.2 ± 2.1	18.3	1.6	6.5 ± 4.8	5.8	**-3.5**	-1.4 ± 2.0
2	**26.6**	19.5	24.6 ± 1.9	18.0	2.5	6.9 ± 4.4	2.8	-2.8	-1.2 ± 1.5
3	27.2	19.1	24.9 ± 2.3	17.6	1.8	6.4 ± 4.5	2.1	-3.2	**-2.1 ±1.3**
4	27.8	19.3	**25.2 ± 2.4**	17.8	1.6	6.3 ± 4.7	5.6	-3.0	-1.4 ± 1.9
5	27.2	17.5	24.5 ± 2.9	16.1	1.3	5.3 ± 4.2	**1.9**	-2.9	-1.5±1.4
6	27.0	15.1	22.6 ± 3.6	13.8	1.1	4.2 ± 3.7	**1.9**	-2.5	-1.1± 1.0
7	**28.6**	19.5	25.1 ± 2.4	18.0	2.6	6.9 ± 4.4	4.8	-2.8	-1.2±1.9
8	27.7	20.1	25.0 ± 2.1	**18.9**	**2.9**	**8.3 ± 4.6**	2.7	-3.2	-1.6 ± 1.5
9	28.0	17.8	24.7 ± 3.0	16.4	2.0	6.1 ± 4.1	2.1	-3.1	-1.6 ± 1.4
10	27.8	18.8	24.8 ± 2.7	17.5	2.5	7.4 ± 4.3	5.4	**-3.6**	-1.5± 2.0
11	27.7	18.9	24.7± 2.5	17.6	2.2	6.9 ± 4.4	2.8	-2.9	-1.4 ± 1.6
12	27.7	15.5	23.7 ± 3.9	14.3	1.6	5.2 ± 3.6	2.0	-3.2	-1.8± 1.4
13	27.6	14.8	23.0 ± 4.1	13.5	1.2	**4.1 ± 3.7**	5.1	-3.4	-1.6± 2.0
14	27.4	14.3	**22.1 ± 3.9**	13.0	1.7	4.3 ± 3.4	2.2	-3.3	-1.1± 1.5
15	27.6	**13.8**	22.7 ± 4.7	**12.8**	1.7	4.7 ± 3.4	**6.9**	**0.4**	**3.9 ± 2.0**
16	27.9	18.1	24.3 ± 2.8	16.9	1.8	6.6 ± 4.4	4.4	-2.9	-1.3 ± 1.8
17	27.6	16.6	24.2 ± 3.4	15.3	**1.0**	4.9 ± 4.2	2.3	**-3.5**	-2.0 ± 1.4
18	28.4	19.0	24.9 ± 2.6	17.7	2.0	6.9 ± 4.6	2.2	-2.4	-1.5 ± 1.2

Temperature highlighted with red color font indicated the highest temperature and the blue color indicated the lowest temperature

Table 4.3 Temperature survey result for smoky bite chicken sausage survey number 2 (2 days of storage)

No. of data logger	Packaging Room			Ice slush tank (cooling process)			Chill storage		
	Max temperature (°C)	Min temperature (°C)	Mean temperature (°C)	Max temperature (°C)	Min temperature (°C)	Mean temperature (°C)	Max temperature (°C)	Min temperature (°C)	Mean temperature (°C)
1	28.0	15.3	22.8 ± 3.6	14.0	1.1	4.9 ± 3.6	3.2	-2.7	-0.8 ± 1.2
2	28.8	17.3	24.0 ± 3.2	16.0	1.1	5.7 ± 4.3	2.0	-2.8	-1.0 ± 1.0
3	28.0	18.2	24.0 ± 2.7	16.9	1.4	6.4 ± 4.6	1.7	-3.1	-1.6 ± 1.0
4	27.3	13.5	21.5 ± 4.6	12.3	1.3	4.5 ± 3.3	3.8	-3.4	-1.1 ± 1.5
5	27.0	16.0	23.2 ± 3.3	14.7	0.6	4.7 ± 4.1	3.3	-3.5	-1.3 ± 1.4
6	26.8	15.1	22.6 ± 3.6	13.8	0.8	4.7 ± 3.8	1.9	-2.4	-1.1 ± 1.0
7	25.8	16.3	23.1 ± 3.0	15.0	1.4	5.4 ± 3.9	**3.9**	-2.7	**0.5 ± 1.4**
8	26.1	14.5	22.2 ± 3.8	13.2	**1.0**	4.5 ± 3.5	**1.5**	-3.2	-1.8 ± 0.9
9	**31.6**	**31.3**	**31.5 ± 0.1**	**31.2**	**2.8**	**22.5 ± 10.4**	2.8	**-2.3**	-0.8 ± 3.2
10	26.8	13.7	21.6 ± 4.2	12.4	1.2	**4.2 ± 3.3**	4.2	-2.3	0.0 ± 1.3
11	26.5	15.1	22.6 ± 3.6	14.0	1.3	5.1 ± 3.7	2.0	-2.4	-0.7 ± 1.0
12	25.6	15.2	22.3 ± 3.4	13.9	1.2	4.9 ± 3.7	1.7	-2.7	-1.5 ± 0.9
13	**25.3**	14.2	21.7 ± 3.7	12.9	1.1	4.6 ± 3.4	3.6	-3.9	-1.6 ± 1.7
14	25.4	14.3	22.0 ± 3.8	13.0	1.3	4.7 ± 3.4	2.1	-3.5	-1.2 ± 1.5
15	25.5	14.7	22.1 ± 3.5	13.4	1.1	4.7 ± 3.6	2.1	**-4.0**	-1.6 ± 1.5
16	26.2	14.3	22.0 ± 3.8	13.1	1.3	4.7 ± 3.5	2.6	-3.4	-1.2 ± 1.5
17	25.5	15.2	22.2 ± 3.3	14.1	1.3	5.3 ± 3.8	1.8	-3.9	**-1.9 ± 1.2**
18	26.5	**13.1**	**21.2 ± 4.3**	**12.0**	1.4	4.3 ± 3.1	1.9	-3.7	-1.7 ± 1.3

Temperature highlighted with red color font indicated the highest temperature and the blue color indicated the lowest temperature

The survey result showed the highest mean and maximum temperature in survey number 3 was located in the front shelf upper level (DL7) on the right side of the chiller. The lowest mean, maximum and minimum temperature was located in the back shelf lower level (DL3) on the right side of the chiller (Table 4.4). In survey number 4, the result showed the highest mean temperature during chill storage was located in middle shelf upper level (DL4) on the right side of the chiller.

The lowest mean temperatures were located at back shelf lower level (DL3) on the right side and at middle shelf lower level (DL15) on the left side in the chiller. The highest maximum temperatures were found at back shelf upper level (DL10) and at middle shelf upper level (DL13) on the left side of the chiller. The lowest maximum temperatures were located in front shelf at middle (DL17) and lower level (DL18) on the left side in the chiller. The highest minimum temperature was located in middle shelf center level (DL5) and the lowest minimum temperature was in same shelf lower level (DL6) on the right side of the chiller (Table 4.5).

Survey number 5 showed that the highest mean temperatures located at back shelf upper level (DL10) on the left side and at front shelf upper level (DL7) on the right side in the chiller. The lowest mean temperature was located at back shelf lower level (DL12) on the left side in the chiller. The highest maximum temperature was found at the front shelf upper level (DL7) on the right side and the lowest at front shelf lower level (DL18) on the left side of the chiller. The highest minimum temperatures were located at back shelf center level (DL2) on the right side and at front shelf lower level (DL18) on the left side in the chiller. The lowest minimum temperatures located at back shelf lower level (DL12) on the left side, middle shelf lower level on the both side (DL6, DL15) of the chiller (Table 4.6).

The result showed the highest mean temperatures located at middle shelf upper level (DL13) on the left side and at front shelf upper level (DL7) on the right side in survey number 6. The lowest mean temperatures located at back shelf lower level (DL12) and at middle shelf lower level (DL15) on the left side in the chiller. The highest maximum temperatures were found at back shelf upper level (DL10) and at middle shelf upper level (DL13) on the left side in the chiller.

The lowest maximum temperature located at front shelf lower level (DL9) on the right side of the chiller. The highest minimum temperatures located at front shelf upper level on the right side (DL7), middle shelf center level (DL14) and at front shelf upper level (DL16) on the left side of the chiller. The lowest minimum temperatures located at back shelf lower level (DL12) on the left side of the chiller (Table 4.7).

Table 4.4 Temperature survey result for corn cheese chicken sausage survey number 3

No. of data logger	Packaging Room			Ice slush tank (cooling process)			Chill storage		
	Max temperature (°C)	Min temperature (°C)	Mean temperature (°C)	Max temperature (°C)	Min temperature (°C)	Mean temperature (°C)	Max temperature (°C)	Min temperature (°C)	Mean temperature (°C)
1	29.7	20.5	**26.0 ± 1.9**	19.0	1.9	6.9 ± 5.1	5.9	-3.5	-1.0 ± 1.8
2	30.1	22.9	26.5 ± 1.5	21.5	3.2	8.6 ± 5.6	3.6	-3.7	-1.4 ± 1.5
3	**29.0**	20.7	26.4 ± 1.8	19.1	**1.8**	6.9 ± 5.1	**1.9**	**-4.1**	**-2.6 ± 0.8**
4	30.5	22.4	26.9 ± 1.6	20.8	3.2	7.9 ± 5.3	8.1	-3.5	-0.4 ± 2.1
5	30.4	21.8	**26.0 ± 1.7**	20.2	2.0	7.3 ± 5.4	3.7	-3.1	-1.0 ± 1.3
6	30.0	22.8	26.6 ± 1.5	21.4	2.4	8.3 ± 5.7	2.3	-3.5	-2.2 ± 0.8
7	**31.9**	**23.5**	**27.3 ± 1.6**	**22.1**	3.2	8.7 ± 5.8	**12.1**	-2.4	**1.4 ± 2.7**
8	31.2	23.0	26.3 ± 1.5	21.7	3.5	**9.2 ± 5.6**	4.8	-3.4	-0.8 ± 1.6
9	30.5	23.2	26.9 ± 1.5	21.8	3.2	8.7 ± 5.8	5.2	-3.4	-0.8 ± 1.8
10	30.6	22.4	26.8 ± 1.6	20.9	3.1	8.1 ± 5.5	7.5	-2.7	-0.6 ± 2.0
11	30.6	21.0	26.5 ± 1.9	19.5	2.7	7.1 ± 5.1	4.9	-3.4	-1.0 ± 1.6
12	29.7	21.2	**26.0 ± 1.6**	19.8	2.6	7.7 ± 5.3	2.7	-3.2	-1.7 ± 1.2
13	29.8	21.1	26.5 ± 1.8	19.7	3.1	7.6 ± 5.1	4.8	-3.3	-0.9 ± 1.5
14	31.5	21.6	26.6 ± 1.9	20.2	3.5	8.1 ± 5.1	3.7	-3.4	-0.8 ± 1.3
15	30.2	22.8	26.5 ± 1.4	21.4	2.4	8.3 ± 5.7	2.3	-3.2	-2.2 ± 0.8
16	30.8	21.9	26.6 ± 1.7	20.5	2.9	8.0 ± 5.4	5.4	-2.6	-0.1 ± 1.7
17	30.0	**19.7**	26.1 ± 2.1	**18.0**	2.5	**6.2 ± 4.7**	6.3	-3.0	-0.3 ± 1.7
18	30.5	21.7	26.6 ± 1.7	20.3	**4.0**	9.0 ± 4.9	3.9	**-2.2**	-1.0 ± 1.1

Temperature highlighted with red color font indicated the highest temperature and the blue color indicated the lowest temperature

Table 4.5 Temperature survey result for squid chicken sausage survey number 4 (7 days of storage)

No. of data logger	Packaging Room			Ice slush tank (cooling process)			Chill storage		
	Max temperature (°C)	Min temperature (°C)	Mean temperature (°C)	Max temperature (°C)	Min temperature (°C)	Mean temperature (°C)	Max temperature (°C)	Min temperature (°C)	Mean temperature (°C)
1	30.4	19.4	24.6 ± 2.4	17.9	1.7	6.8 ± 4.7	8.3	-4.0	-1.2 ± 2.1
2	29.6	**24.2**	25.9 ± 1.4	**23.3**	2.3	9.2 ± 6.4	5.6	-4.0	-1.6 ± 1.7
3	30.0	23.3	24.9 ± 1.8	22.6	2.6	9.5 ± 6.1	4.6	-4.6	-2.6 ± 1.5
4	29.5	22.6	25.2 ± 2.0	21.5	2.5	8.9 ± 5.7	8.1	-3.2	-0.5 ± 1.9
5	30.2	17.0	23.4 ± 4.3	17.0	**1.2**	5.7 ± 4.6	6.5	-3.0	-0.9 ± 1.6
6	30.0	17.6	24.0 ± 3.0	16.3	1.6	6.3 ± 4.3	4.1	**-4.9**	**-2.9 ± 1.3**
7	29.8	16.4	23.8 ± 3.4	15.1	1.8	5.7 ± 3.9	8.6	-3.9	-1.2 ± 2.1
8	28.6	22.9	25.2 ± 1.6	21.8	2.8	9.1 ± 5.6	4.1	-3.9	-1.8 ± 1.5
9	29.5	21.6	24.9 ± 1.9	20.4	2.1	8.0 ± 5.3	4.4	-4.1	-2.3 ± 1.4
10	29.3	22.9	25.7 ± 1.5	21.8	3.0	9.5 ± 5.6	**9.3**	-3.6	-0.8 ± 2.3
11	30.8	17.5	24.8 ± 3.7	16.1	2.8	6.0 ± 4.2	6.5	-3.5	-1.3 ± 1.7
12	30.3	18.2	24.7 ± 3.2	16.6	1.8	5.9 ± 4.4	4.8	-4.1	-2.2 ± 1.5
13	**27.4**	18.3	**23.3 ± 2.5**	16.8	**1.2**	5.8 ± 4.5	**9.3**	-4.1	-1.1 ± 2.2
14	**30.7**	**14.3**	24.1 ± 4.8	**12.9**	2.5	**4.3 ± 3.3**	6.5	-3.2	-0.9 ± 1.6
15	29.7	23.0	25.7 ± 1.7	21.8	2.3	8.7 ± 5.8	4.3	-4.6	-2.6 ± 1.5
16	30.6	23.5	26.0 ± 2.0	22.6	**4.0**	**10.8 ± 5.7**	6.0	-3.4	-0.7 ± 1.8
17	29.5	23.8	25.5 ± **1.5**	22.8	3.9	10.2 ± 5.6	**3.7**	-3.9	-1.3 ± 1.7
18	29.4	23.3	**26.1 ± 1.6**	22.1	2.8	9.4 ± 5.8	**3.7**	-3.8	-1.1 ± 1.5

Temperature highlighted with red color font indicated the highest temperature and the blue color indicated the lowest temperature

Table 4.6 Temperature survey result for corn cheese chicken sausage survey number 5 (7 days of storage)

No. of data logger	Packaging Room			Ice slush tank (cooling process)			Chill storage		
	Max temperature (°C)	Min temperature (°C)	Mean temperature (°C)	Max temperature (°C)	Min temperature (°C)	Mean temperature (°C)	Max temperature (°C)	Min temperature (°C)	Mean temperature (°C)
1	27.5	22.3	24.2 ± 1.4	21.7	2.3	9.1 ± 5.8	8.5	0.2	2.2 ± 1.7
2	**25.9**	21.9	24.0 ± 1.2	20.5	1.5	7.8 ± 5.8	4.4	**0.8**	0.4 ± 1.0
3	27.1	17.8	**23.4 ± 2.0**	16.5	1.1	5.8 ± 4.5	3.2	-1.4	-0.2 ± 0.8
4	27.4	22.7	24.7 ± 1.4	24.3	**3.6**	**11.6 ± 6.4**	8.5	0.0	1.7 ± 1.4
5	27.0	**16.8**	23.8 ± 2.6	**15.6**	1.6	6.1 ± 4.1	3.8	0.7	0.5 ± 0.8
6	26.9	20.4	23.7 ± 1.3	19.2	1.4	7.3 ± 5.3	6.5	**-1.6**	1.4 ± 1.7
7	26.2	20.0	24.3 ± 1.6	18.8	1.8	7.5 ± 5.0	**9.0**	0.1	2.3 ± 1.5
8	27.1	18.5	23.9 ± 1.8	17.1	1.2	6.4 ± 4.7	5.0	0.4	1.1 ± 0.9
9	26.2	17.9	23.6 ± 2.1	16.6	1.1	5.9 ± 4.6	3.3	0.4	0.9 ± 0.7
10	27.3	21.6	24.4 ± 1.3	20.1	1.5	7.3 ± 5.5	8.8	0.1	**2.6 ± 1.6**
11	26.8	20.2	24.7 ± 1.8	18.9	1.8	7.4 ± 5.1	8.5	0.1	2.0 ± 1.3
12	26.0	22.3	24.6 ± 1.4	23.4	2.3	9.6 ± 6.4	3.7	**-1.6**	**-0.5 ± 0.9**
13	26.0	22.3	24.1 ± 1.3	20.9	2.0	8.6 ± 5.6	7.4	0.6	1.6 ± 1.4
14	26.2	**22.8**	24.4 ± 1.1	22.5	2.1	9.2 ± 6.2	4.3	-1.0	0.3 ± 0.9
15	27.0	17.2	24.1 ± 2.4	15.9	**1.0**	**5.7 ± 4.3**	3.9	**-1.6**	-0.4 ± 0.9
16	**27.8**	22.6	24.8 ± 1.4	24.3	2.7	11.1 ± 6.9	6.2	0.4	1.5 ± 1.2
17	**27.8**	22.7	**25.1 ± 1.5**	**24.4**	2.1	9.6 ± 6.8	3.8	0.7	0.5 ± 0.8
18	26.2	22.5	24.5 ± 1.2	23.1	2.5	9.6 ± 6.3	**3.1**	**0.8**	0.1 ± 0.7

Temperature highlighted with red color font indicated the highest temperature while the blue color indicated the lowest temperature

Table 4.7 Temperature survey result for hotdog chicken sausage survey number 6 (7days of storage)

No. of data logger	Packaging Room			Ice slush tank (cooling process)			Chill storage		
	Max temperature (°C)	Min temperature (°C)	Mean temperature (°C)	Max temperature (°C)	Min temperature (°C)	Mean temperature (°C)	Max temperature (°C)	Min temperature (°C)	Mean temperature (°C)
1	**29.7**	**24.6**	**27.5 ± 1.1**	**23.9**	3.1	10.0 ± 5.9	4.1	-3.6	-1.4 ± 1.6
2	28.2	22.0	26.4 ± 1.7	21.2	2.9	8.7 ± 5.1	2.8	-3.9	-2.2 ± 1.3
3	28.5	22.8	26.6 ± 1.5	22.0	3.7	9.8 ± 5.0	3.6	**-4.6**	-2.9 ± 1.2
4	28.0	23.5	26.8 ± 1.3	22.7	3.6	9.8 ± 5.3	3.6	-3.6	-0.9 ± 1.5
5	28.0	22.1	26.5 ± 1.6	21.2	3.3	9.0 ± 4.9	3.2	-3.9	-1.6 ± 1.3
6	27.6	23.1	26.4 ± 1.3	22.4	2.5	9.3 ± 5.6	2.4	-4.3	-3.1 ± 1.0
7	29.3	23.1	26.5 ± 1.4	22.3	2.5	9.3 ± 5.6	4.6	**-3.3**	-0.9 ± 1.7
8	**26.4**	23.5	25.8 ± 0.8	22.8	3.5	9.8 ± 5.4	3.4	-3.4	-1.8 ± 1.2
9	27.9	19.9	25.2 ± 2.3	19.2	2.3	8.0 ± 4.8	**2.2**	-3.4	-1.7 ± 1.0
10	29.2	22.7	26.2 ± 1.5	22.1	**4.5**	**10.7 ± 5.0**	**4.8**	-4.1	-1.6 ± 1.9
11	28.8	22.7	26.6 ± 1.7	21.9	3.9	9.9 ± 5.0	3.8	-4.6	-2.8 ± 1.3
12	29.0	**19.0**	**24.9 ± 2.8**	**18.2**	**2.1**	**7.0 ± 4.4**	2.3	**-5.1**	**-3.8 ± 1.1**
13	28.0	22.2	25.6 ± 1.5	21.4	3.5	9.7 ± 5.0	**4.8**	-3.4	**-0.8 ± 1.7**
14	28.6	20.0	**24.9 ± 2.2**	19.2	2.4	7.8 ± 4.6	2.4	**-3.3**	-1.4 ± 1.1
15	**29.7**	21.0	25.4 ± 2.0	20.2	2.4	7.8 ± 4.9	2.3	-4.5	**-3.8 ± 0.8**
16	28.7	22.2	25.4 ± 1.5	21.4	3.8	9.7 ± 4.9	3.7	**-3.3**	-1.5 ± 1.3
17	28.6	21.1	25.5 ± 1.9	20.3	2.8	8.4 ± 5.4	2.7	-3.8	-1.9 ± 1.1
18	29.0	23.2	25.9 ± 1.4	22.5	3.3	10.2 ± 4.8	3.2	-4.0	-2.3 ± 1.2

Temperature highlighted with red color font indicated the highest temperature and the blue color indicated the lowest temperature

From all the surveys conducted in the study, it seems that high temperature most likely located in upper level of the shelves and low temperature was in the lower level of the shelves in chiller. The reason was the thermal behavior of cold air and the circulation system in the chiller. Since the cooling unit is installed in the bottom at the back of the chiller room, cold air is circulated from lower level at the back to lower level front and flows back from upper level front to upper level back of the chiller (Figure 4.5). Therefore, low temperature is likely found at lower level of the shelf compared to upper level and center level in the chiller while warmer air is on the top level of the shelves in the chiller.

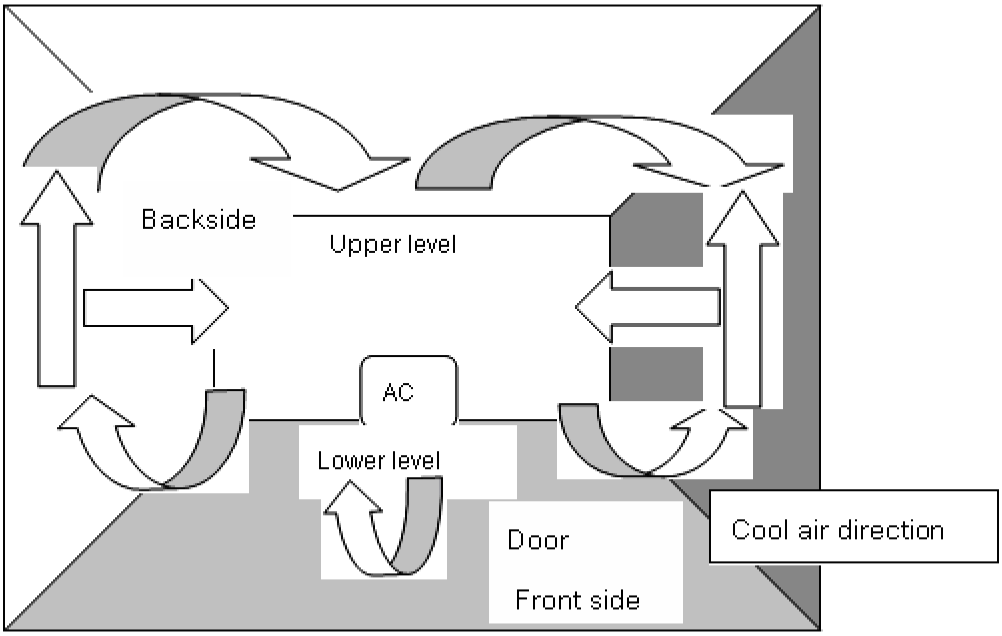


Figure 4.5 Air circulation in chiller

This refrigerated system circulates the cold air over the system's evaporator. The evaporator of cooling system was placed in lower level at the backside of the chiller. Movement of air is by mechanical fans and gravitational force that relies on density being greater for cold air than for warm air (Dennis and Stringer, 1992). The airflow distribution in the chiller is dependent on the produce, cooling medium, the geometry and characteristic of the chiller (Hoang et. al., 2000). A minimum cooling time of approximately 2 hours was required to cool warm products to appropriate chill temperature (Salvadori and Mascheroni, 2005).

The result showed that the temperature fluctuation and variation occurred mostly during daytime and working day (Figure 4.6 and Figure 4.8). The temperature was lower and also stable on non-work days compare to working day (Figure 4.7 and Figure 4.9). This condition was due to frequent door opening during loading and unloading activity of sausages into and out of the chiller. The front shelves on the top part of the chiller mostly experienced warm air during door opening. Normally, temperature fluctuation occurred during daytime in the working day started from 4.00 pm until 9.00 pm especially on Monday, Tuesday and Friday when unloading for distribution took place (Figure 4.6). The temperature fluctuated more in the front shelf where warm air from outside the chiller mostly easily interrupted with cool air in the chiller (Figure 4.4 and figure 4.6). During loading activity the temperature shot up to 12.1°C when the door opened more than an hour which caused the interruption of cold chain (Figure 4.6).

Cold chain is considered interrupted when the temperature exceeds 5°C in chill storage (Billiard, 2003). Temperatures for perishable foods like meat and poultry products were highly affected on their quality even in small temperature fluctuations could lead the physico-chemical and microbial quality (Wuo and Luo, 2011).

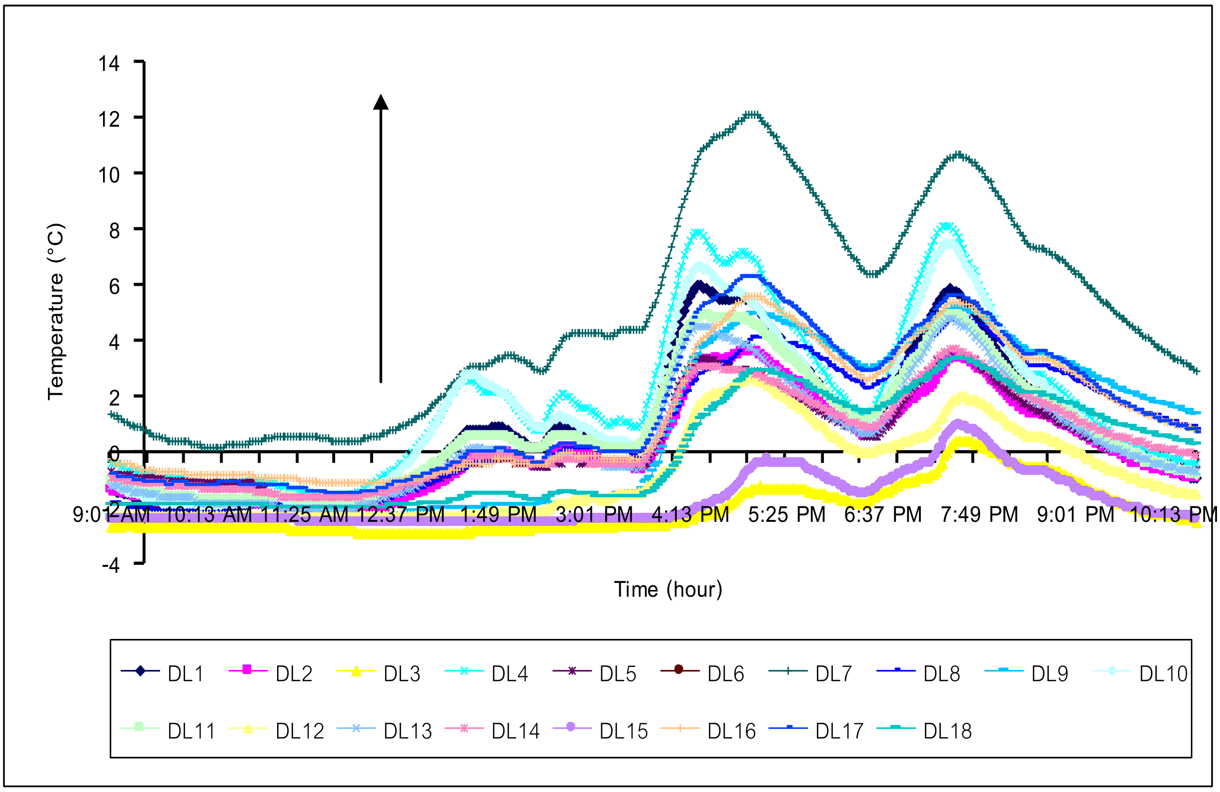


Figure 4.6 Example temperature profiles during chilled storage in working day in survey number 3; DL: Data logger

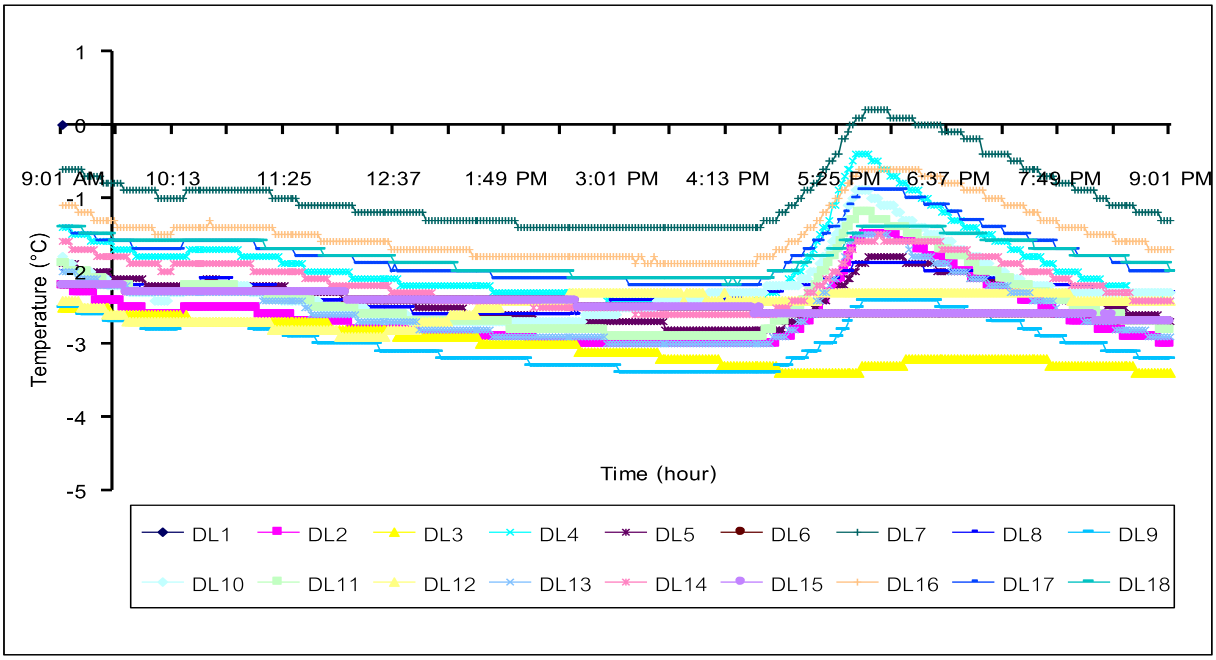


Figure 4.7 Example temperature profiles during chilled storage on non-working day in survey number 3; DL: Data logger

In any chiller, entrances are required for loading finished products and they are a major source of heat infiltration. Air infiltration could account for more than half the total heat load for refrigerator. Infiltration of warm moist air through doorways into refrigerated rooms during loading products causes many problems such as increased costs for running and defrosting the refrigeration system (Foster, 2007). Azzouz et al. (1993) measured a heat infiltration of 3.4% between cold store and ambient during door opening. Cold air usually leaves at the bottom and warm moist air enters at the top.

Temperature fluctuation frequently occurred during loading the sausages from chiller to vehicle for transportation to retailer. Example of the worst temperature condition during chill storage is shown in Figure 4.8. The temperature jumped up to 12.1°C from 4°C within 84 minutes during loading activity. To bring the temperature down to normal controlled temperature below 5°C took about 90 minutes (the first peak in Figure 4.8).

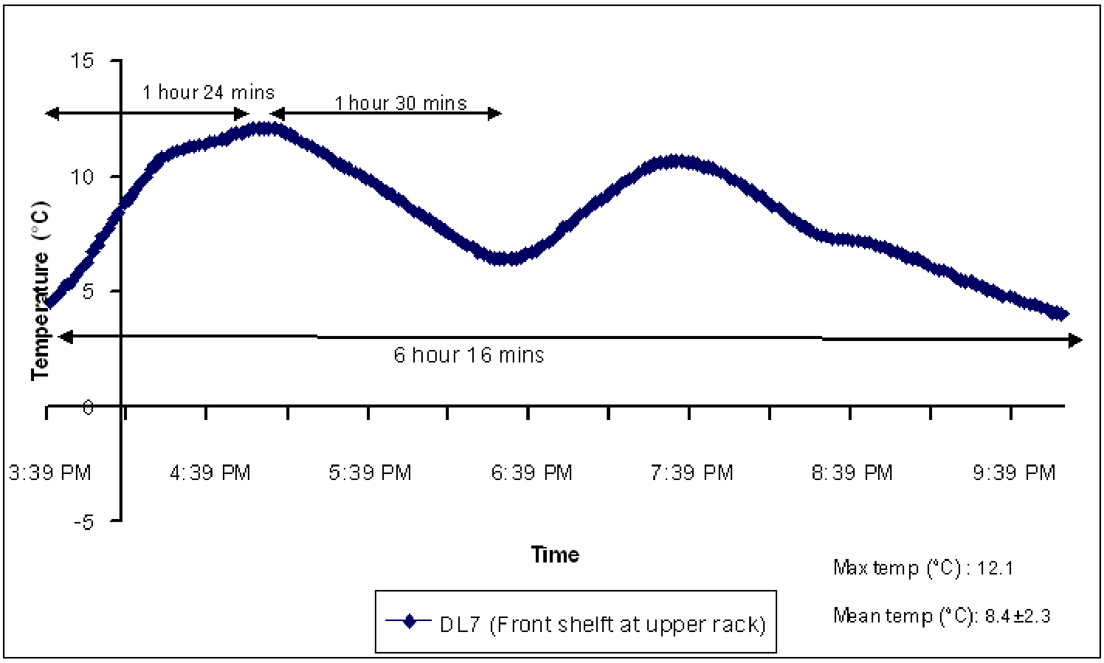


Figure 4.8 Whole section worst temperature during chill storage survey no. 3 on working day in front shelf upper level (DL7)

4.1.2 Microbiological quality of chicken sausages after chilled storage

The results showed that after the sausages were cooked, the mean total TPC was less than 10 cfu/g and mean TPC after casing removal was in the 3.7 to 4.2 log cfu/g range (Table 4.8). TPC was high after peeling off the casing of sausages due to possible contamination through the peeling machine that was not frequently cleaned. The frequency of TPC greater than 5 log cfu/g, which exceeds the microbiological limit recommended by Thai Industrial Standards Institute for meat sausage, of packed sausages after chill storage was 2 from the total of 108 packs of sausages in this survey. The highest TPC count was found in DL12 (4.23 log of cfu/g) located at the back shelf lower level on the left side of the chiller in survey number 1 for two days of storage (Table 4.8). The lowest TPC count was found in DL17 (3 log of cfu/g) located at front shelf center level on left side chiller (Table 4.3).

The highest TPC in batch 2 was 5.25 log in DL11 located in middle shelf center level on the left side of the chiller. The lowest count was in DL2 (2 log of cfu/g) located at back shelf center level on the right side of the chiller. The result showed the highest TPC in survey number 3 in DL5 (5 log of cfu/g) located at middle shelf center level on the right side of the chiller for 7 days of storage. The lowest TPC was in DL15 (3.23 log of cfu/g) located at middle shelf lower level on the left side of the chiller. In survey number 4, the highest TPC was in DL1 (4.17 log of cfu/g) located at back shelf upper level on the right side in the chiller. The lowest TPC was found in DL18 located at front shelf lower level on the left side of the chiller. The highest TPC in survey number 5 was in DL6 (4.43 log of cfu/g) located at middle shelf lower level on the right side chiller. The lowest TPC were in DL17 and DL18 (3.17 log of cfu/g) located at front shelf at center and lower level on the left side in chiller. The result showed the highest TPC count in survey number 6 was in DL14 (4.4 log of cfu/g) located at middle shelf center level on the left side of the chiller.

The lowest TPC count was in DL1 (3.19 log of cfu/g) located at back shelf upper level on the right side of chiller (Table 4.8). There was not much different in TPC count during two days and seven days chill storage. It is because the temperature during storage was low and most of the temperatures reach freezing temperature (0°C to -5 °C) (Table 4.2 to 4.7). However, the results showed the TPC count in 7 days of storage was slightly high in certain type of sausage. In survey number 6, the results showed that 50% of chicken hotdog had TPC above 4 log cfu/g (Table 4.9). However, there were several locations where the mean and minimum temperatures were low but the TPC count was high. It might be due to (1) high number of temperature variation or frequent temperature fluctuation during storage and (2) higher initial contamination in those samples possibly due to case peeling process mention above.

Table 4.8 Total plate count (TPC) of cooked and peeled chicken sausages

Chicken sausage	Mean log cfu/g
After cook	< 10 cfu/g
After casing removal	3.7 to 4.2

Table 4.9 Total plate count (TPC) and mean temperature of chicken sausages after chill storage

Data logger no.	**Survey 1**		**Survey 2**		**Survey 3**		**Survey 4**		**Survey 5**		**Survey 6**	
	log cfu/g	Mean temperature (°C)	log cfu/g	Mean temperature (°C)	log cfu/g	Mean temperature (°C)	log cfu/g	Mean temperature (°C)	log cfu/g	Mean temperature (°C)	log cfu/g	Mean temperature (°C)
1	3.23	-1.4 ± 2.0	3.20	-0.8 ± 1.2	4.28	-1.0 ± 1.8	**4.17**	-1.2 ± 2.1	4.29	2.2 ± 1.7	3.19	-1.4 ± 1.6
2	3.69	-1.2 ± 1.5	2.00	-1.0 ± 1.0	4.11	-1.4 ± 1.5	3.26	-1.6 ± 1.7	4.09	0.4 ± 1.0	3.33	-2.2 ± 1.3
3	3.25	**-2.1 ±1.3**	2.90	-1.6 ± 1.0	3.99	**-2.6 ± 0.8**	3.34	-2.6 ± 1.5	4.37	-0.2 ± 0.8	4.18	-2.9 ± 1.2
4	4.11	-1.4 ± 1.9	3.18	-1.1 ± 1.5	4.25	-0.4 ± 2.1	3.66	-0.5 ± 1.9	4.41	1.7 ± 1.4	4.18	-0.9 ± 1.5
5	3.32	-1.5±1.4	2.94	-1.3 ± 1.4	**5.00**	-1.0 ± 1.3	3.29	-0.9 ± 1.6	4.15	0.5 ± 0.8	4.33	-1.6 ± 1.3
6	3.36	-1.1± 1.0	3.01	-1.1 ± 1.0	3.42	-2.2 ± 0.8	3.10	**-2.9 ± 1.3**	**4.43**	1.4 ± 1.7	3.27	-3.1 ± 1.0
7	4.01	-1.2±1.9	2.93	**0.5 ± 1.4**	3.31	**1.4 ± 2.7**	3.19	-1.2 ± 2.1	4.36	2.3 ± 1.5	4.39	-0.9 ± 1.7
8	3.28	-1.6 ± 1.5	3.01	-1.8 ± 0.9	3.48	-0.8 ± 1.6	3.24	-1.8 ± 1.5	3.36	1.1 ± 0.9	4.19	-1.8 ± 1.2
9	3.23	1.6 ± 1.4	3.12	0.8 ± 3.2	3.41	0.8 ± 1.8	3.35	2.3 ± 1.4	3.21	0.9 ± 0.7	4.16	1.7 ± 1.0
10	4.15	-1.5± 2.0	3.23	0.0 ± 1.3	3.31	-0.6 ± 2.0	3.12	-0.8 ± 2.3	3.38	**2.6 ± 1.6**	4.10	-1.6 ± 1.9
11	3.17	-1.4 ± 1.6	**5.25**	-0.7 ± 1.0	4.39	-1.0 ± 1.6	3.24	-1.3 ± 1.7	4.24	2.0 ± 1.3	3.47	-2.8 ± 1.3
12	**4.23**	-1.8± 1.4	2.97	-1.5 ± 0.9	4.35	-1.7 ± 1.2	3.06	-2.2 ± 1.5	4.08	**-0.5 ± 0.9**	3.50	**-3.8 ± 1.1**
13	3.94	-1.6± 2.0	2.59	-1.6 ± 1.7	3.66	-0.9 ± 1.5	3.66	-1.1 ± 2.2	3.22	1.6 ± 1.4	4.29	**-0.8 ± 1.7**
14	3.96	-1.1± 1.5	2.71	-1.2 ± 1.5	3.29	-0.8 ± 1.3	3.29	-0.9 ± 1.6	3.22	0.3 ± 0.9	**4.40**	-1.4 ± 1.1
15	3.04	**3.9 ± 2.0**	2.69	-1.6 ± 1.5	3.23	-2.2 ± 0.8	3.23	-2.6 ± 1.5	3.18	-0.4 ± 0.9	4.28	**-3.8 ± 0.8**
16	3.25	-1.3 ± 1.8	2.63	-1.2 ± 1.5	4.40	-0.1 ± 1.7	3.44	-0.7 ± 1.8	3.21	1.5 ± 1.2	4.33	-1.5 ± 1.3
17	3.00	-2.0 ± 1.4	2.59	**-1.9 ± 1.2**	4.14	-0.3 ± 1.7	3.11	-1.3 ± 1.7	3.17	0.5 ± 0.8	4.22	-1.9 ± 1.1
18	3.93	-1.5 ± 1.2	2.99	-1.7 ± 1.3	3.41	-1.0 ± 1.1	2.92	-1.1 ± 1.5	3.17	0.1 ± 0.7	4.04	-2.3 ± 1.2

The red font highlighted the highest log of TPC and the blue font highlighted the lowest log TPC in each survey

Under refrigeration temperature below 5°C, psychotropic bacteria are able to multiply and cause spoilage. However, the mesophilic bacteria that initially predominate on the products remain the same or decrease in number (Russel, 2000). Lowering the temperature during chill storage below freezing point tremendously decreased the microbial growth. However, lowering the temperature into the freezing zone during storage will cause adverse affect on the sausages texture. At the same time, too low storage temperature increases the operational cost (electricity and utility).

Walker and Betts (2000) recommended that foods permitting growth of microorganisms should be stored at lower than 10ºC and preferably about 4ºC to prevent the growth of pathogens or toxin production. Foster (2007) reported that the effect of chilled storage life was more pronounced and was halved for temperature below 10°C at each 2°C to 3°C risen. In the normal temperature range of -1.5°C and 5°C for chilled meat, there can be as much as an eightfold difference in growth rate between the upper and lower temperatures. Frequent door openings of the chiller also have a critical impact on product temperature and storage life (Foster, 2007).

Low refrigeration temperatures could restrain the growth of several spoilage microorganisms. Storage and distribution at low temperature as well as packaging are two of the most regularly encountered methods of preservation for microbiologically unstable products (Pothakos, Samapundo and Devlieghere, 2012). A reduction in the average storage temperature could increase the length of retention and quality of many chilled foods. Bacteria counts will decrease, leading to an increase in safety and shelf life of the product (Foster 2007). Mesophilic and psychrotrophic counts were significantly higher in processed chicken products like sausages than in chicken parts and these results indicated cross contamination and temperature abuse during processing, storage and displaying of product (Astorga et al., 2002).

Hence, controlling finished products at chill temperatures could reduce the number of microbial and ensure the safety of the chilled foods during chill storage.

4.1.3 Physico-chemical properties of chicken sausages after chilled storage

4.1.3.1 pH and titratable acidity

The results showed that the pH and the titratable acidity of the sausages varied in narrow range in the same batch for all the surveys. In survey number 1 the pH value varied in a narrow range of 6.86 to 6.97, for survey number 2 the pH range of 6.52 to 6.81, for survey number 3 pH was ranged from 6.72 to 7.12, for survey number 4 pH value ranged from 6.32 to 6.46 for survey number 5 the pH ranged from 6.73 to 6.84 and for survey number 6 the pH varied from 6.67 to 6.79 (Table 4.10). In addition, the titratable acidity in the same survey varied in a narrow range of 0.017% to 0.028% for survey number 1, 0.019% to 0.040% for survey number 2, 0.015% to 0.045% for survey number 3, 0.054% to 0.069% for survey number 4, 0.024% to 0.032% for survey number 5 and 0.044% to 0.053% for survey number 6.

The differences of pH and acidity between surveys stemmed from the fact that the sample differed from one survey to another survey. Little variation in pH and acidity during storage inferred that chill storage of sausages in different shelf position did not affect the quality of the sausages. This was due to very low temperature during storage. It was found that pH and titratable acidity were not related with total plate count (TPC).

There was no relationship between pH and titratable acidity with TPC of chicken sausages after two days chill storage. No difference was found in the pH and the acidity of the samples stored for 2 days and 7 days. Nevertheless, there was relation between pH and acidity in survey number 5 and survey number 6 of 7 days storage where the pH was low and the acidity was high after chill storage (Table 4.10).

Table 4.10 pH and titratable acidity of chicken sausages after chill storage in six surveys

Data	**Survey 1**		**Survey 2**		**Survey 3**		**Survey 4**		**Survey 5**		**Survey 6**	
logger no.	pH	%TA	pH	%TA	pH	%TA	pH	%TA	pH	%TA	pH	%TA
1	6.91	0.021	6.81	0.019	6.80	0.021	6.46	0.063	6.73	0.029	6.67	0.053
2	6.87	0.028	6.79	0.029	6.72	0.015	6.44	0.069	6.73	0.028	6.74	0.044
3	6.93	0.019	6.77	0.026	6.88	0.017	6.43	0.057	6.74	0.027	6.72	0.046
4	6.94	0.023	6.77	0.032	6.76	0.019	6.41	0.057	6.73	0.028	6.74	0.048
5	6.95	0.021	6.75	0.024	6.87	0.018	6.37	0.063	6.79	0.026	6.74	0.046
6	6.97	0.028	6.74	0.026	6.88	0.021	6.32	0.060	6.79	0.026	6.73	0.044
7	6.93	0.018	6.72	0.023	6.83	0.021	6.36	0.063	6.79	0.026	6.68	0.049
8	6.94	0.018	6.68	0.028	7.04	0.018	6.35	0.060	6.78	0.029	6.71	0.046
9	6.91	0.028	6.65	0.025	7.10	0.026	6.40	0.060	6.78	0.032	6.71	0.045
10	6.94	0.024	6.70	0.035	7.12	0.018	6.37	0.066	6.76	0.029	6.71	0.048
11	6.91	0.017	6.69	0.029	6.89	0.022	6.41	0.054	6.75	0.029	6.71	0.051
12	6.89	0.018	6.68	0.033	7.08	0.018	6.38	0.060	6.73	0.032	6.77	0.044
13	6.91	0.020	6.67	0.035	6.95	0.022	6.41	0.057	6.75	0.029	6.73	0.046
14	6.88	0.028	6.63	0.032	6.96	0.045	6.40	0.054	6.74	0.029	6.67	0.053
15	6.86	0.017	6.54	0.040	6.94	0.021	6.43	0.054	6.84	0.024	6.76	0.049
16	6.90	0.019	6.52	0.036	6.97	0.021	6.40	0.063	6.75	0.029	6.79	0.044
17	6.94	0.015	6.56	0.028	7.03	0.018	6.41	0.062	6.76	0.031	6.70	0.049
18	6.88	0.022	6.58	0.035	6.74	0.024	6.40	0.062	6.78	0.024	6.76	0.048

TA unit: % acidity (express as ml of 0.1N NaOH/g), the red font color indicated the lowest pH and the highest acidity in six surveys

Generally, the microbial number increases with time in chilled foods at neutral pH values, low salt concentrations and the absence of preservatives. However, low pH values cause microbial stasis and at chill temperatures so the microorganism may survive for longer periods compared with higher temperatures (Walker and Betts, 2000). Microbial load increases with an increase in final pH in the meat products (Deva and Narayan, 1988).

Lactic acid producing bacteria grows slowly at low temperatures. This group of bacteria is more tolerant of low pH and can multiply at pH as low as 3.6. Lactic acid bacteria usually predominate in vacuum-packed products and modified atmosphere stored foods. Vacuum-packaging provides a favorable environment to microaerophiles, notably the lactic acid bacteria but not for aerobic microorganisms. Lactic acid bacteria are often unable to proliferate at 30°C and the predominant bacterial strains in packaged food products stored at chilling temperature (Pothakos et al., 2012). Spoilage occurrs generally by the production of acid that results in souring (Walker and Betts, 2000). The growth of lactic acid bacteria reduces the quality of products such as vacuum-packaged beef, cooked ring sausages and vienna sausages during extended low temperature storage conditions (Dykes, Cloete and Holy, 1991). In this study, low acid production indicated that less of acidity presence in the sausages due to very low storage temperature which was closed to freezing temperature (0.8°C to -5.1°C). It could be due to less of fermentation of lactic acid bacteria during chilled storage of the sausages.

Dharmaveer, Rajkumar and Mukesh (2007) reported that decrease in pH of meat products was likely due to growth of lactic acid bacteria and depended on carbohydrates content in the meat products. This study was consistent with the study of Santos et. al. (2005) who found that the sausage had a decrease in pH after chilled storage at 4°C in vacuum package. In the other study of Agnihotri and Pal (2000), it was found that the pH of air packed sausages increased during chilled storage. Thomas et al. (2008) found that there was an inverse relationship between titratable acidity and pH where higher titratable acidity causes lower pH in the sausages. Rajani et al. (2007) also reported a reduction in titratable acidity in chicken emulsions stored under refrigeration temperature.

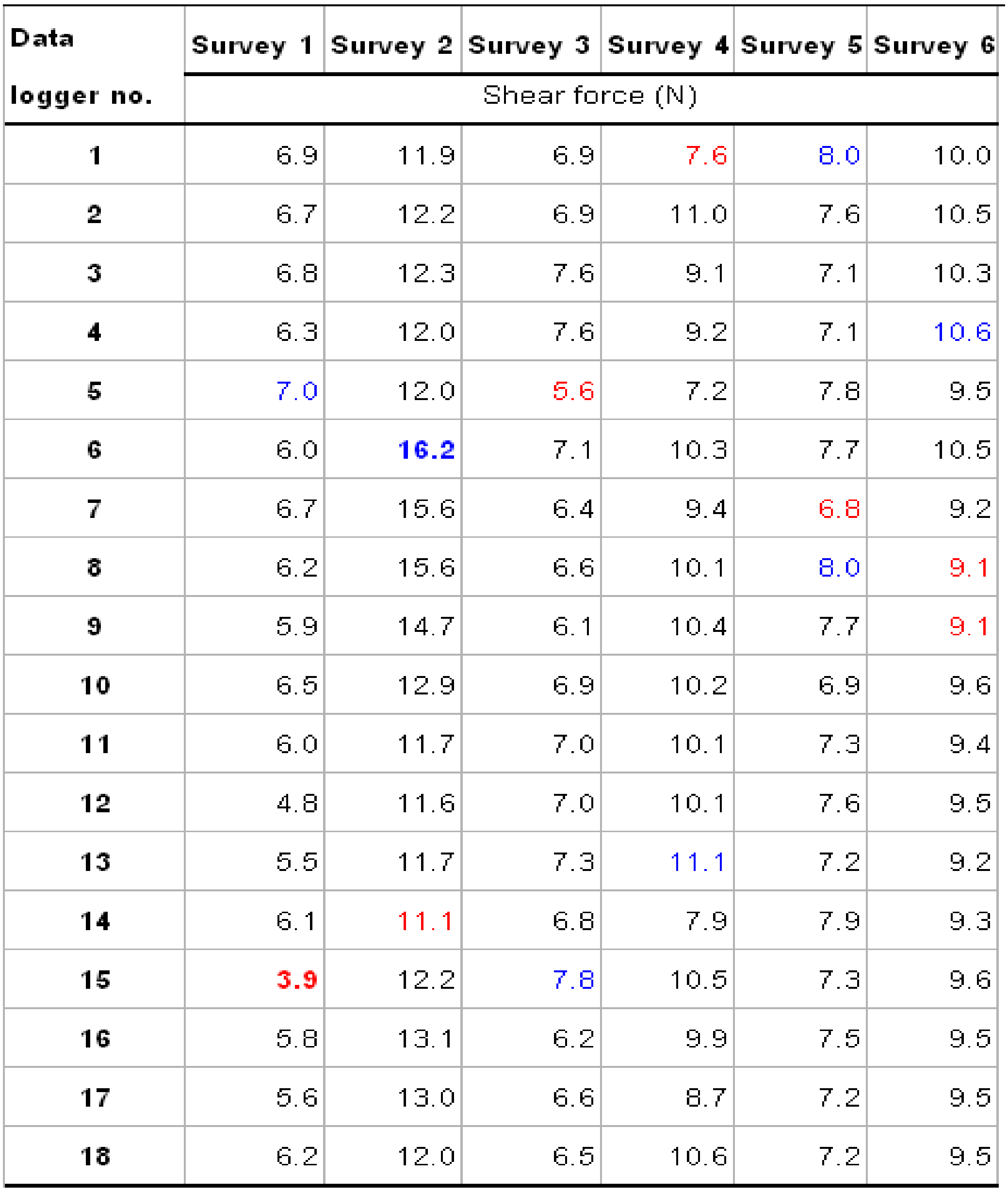
4.1.3.2 Texture

The result showed that the shear force varied in a narrow range between all of the surveys. Shear force value varied in a narrow range of 3.9N to 7.0N for survey number 1, 11.1N to 16.2N for survey number 2, 5.6N to 7.8N for survey number 3, 7.6N to 11.1N for survey number 4, 6.8N to 8.0N for survey number 5 and 9.1N to 10.6N for survey number 6. The difference of shear force values between survey was because the difference in type of sausages and duration of chill storage. There was a relationship between shear force value and TPC of the sausages in different surveys. In survey number 1, the result showed that, high TPC caused spoilage and decreased shear force of the sausages (Table 4.9 and Table 4.11). Likewise, the pH value related to shear force in survey number 1, where the lowest pH indicated spoilage of the sausages causing the texture value to decrease (Table 4.10 and Table 4.11). Similar result was reported by Thomas et al. (2008) that shear force of pork sausages was decreased with decreasing pH due to protein denaturation and subsequent loss of binding properties of meat protein during storage.

The result also showed similar trend in survey number 3, number 4 and number 5 where high TPC caused the decrease in shear force after storage (Table 4.9 and Table 4.11). It might be due to the spoilage when the sausages stored for longer period in chill condition. There was no difference between shear force of the sausages stored for two days and seven days in this survey. There was a relationship between texture and TPC in same type of sausage in survey number 1, number 3 and number 5, eventhough with different durations of chill storage. Higher TPC related to lower shear force in those type of sausages.

Spoilage of sausages was usually caused by the production of acid by gram negative bacteria mostly like lactic acid bacteria (LAB) and this results in souring of the products. Pothakos et al. (2012) reported that the LAB often unable to proliferate at 30°C but favorable the temperature above 5°C and they were the predominant bacterial strains in packaged food products stored at chilled temperature. LAB is the spoilage indicator of the sausages. It was reported that the decrease in pH of meat products were likely due to growth of lactic acid bacteria depend on carbohydrates and protein content in the meat products (Dharmaveer et al., 2007). LAB could produce acids by fermenting carbohydrate that was added in the minced batter caused pH decreased. It was agreed that pH decreased in fresh pork sausages was probably related to the growth of LAB where the initially count was 1 log cfu/g and increased up to 6 log cfu/g during storage at a higher temperature (Chiavaro et al., 2008). Díaz et al. (2008) reported that the LAB count increased to 5.6 log cfu/g when temperature rise more than 8°C in 12 days of storage.

Table 4.11 Texture of the chicken sausages after chill storage of six surveys



Data logger no.	Survey 1	Survey 2	Survey 3	Survey 4	Survey 5	Survey 6
	Shear force (N)					
1	6.9	11.9	6.9	7.6	8.0	10.0
2	6.7	12.2	6.9	11.0	7.6	10.5
3	6.8	12.3	7.6	9.1	7.1	10.3
4	6.3	12.0	7.6	9.2	7.1	10.6
5	7.0	12.0	5.6	7.2	7.8	9.5
6	6.0	**16.2**	7.1	10.3	7.7	10.5
7	6.7	15.6	6.4	9.4	6.8	9.2
8	6.2	15.6	6.6	10.1	8.0	9.1
9	5.9	14.7	6.1	10.4	7.7	9.1
10	6.5	12.9	6.9	10.2	6.9	9.6
11	6.0	11.7	7.0	10.1	7.3	9.4
12	4.8	11.6	7.0	10.1	7.6	9.5
13	5.5	11.7	7.3	11.1	7.2	9.2
14	6.1	11.1	6.8	7.9	7.9	9.3
15	**3.9**	12.2	7.8	10.5	7.3	9.6
16	5.8	13.1	6.2	9.9	7.5	9.5
17	5.6	13.0	6.6	8.7	7.2	9.5
18	6.2	12.0	6.5	10.6	7.2	9.5

The blue font color highlighted the highest shear force while the red font color highlighted the lowest shear force in different surveys of sausages.

Survey number 1, 3 and 5: corn cheese sausage, survey number 2: smoky bite, survey number 4: squid chicken sausage and survey number 6: hot dog chicken

Chilled storage was affected the shear force values of chevon smoke sausages whereas the smoke sausage reduce the moisture content and increased the hardness of the sausages due to low temperature of storage (Dharmaveer et al., 2007). It was also agreed with Fernandez, Rodriguez and Oderiz, (2005) that higher shear force in vacuum-packed sausage was due to moisture content and the low temperature during storage. Similar result in vacuum packed products that reported by Anjaneylu and Kondaiah (1990) for buffalo nuggets in low temperature storage increased the hardness of the products.

4.1.3.3 Color space (L*, a*, b*)

There were four types of sausages that were analyzed on this survey. The types of sausages were as followed:-

Survey number 1: corn cheese (yellowish)
Survey number 2: smoky bite (brownish)
Survey number 3: corn cheese
Survey number 4: squid sausage (white color)
Survey number 5: corn cheese
Survey number 6: hot dog (dark red color)

The results showed that the color parameter of the sausages: L*= lightness, a*= redness and b*= yellowness, were not different in sausages stored at different locations in the chiller (Table 4.12). The L* value in survey number 1 ranged from 61.3 to 63.0, a* value from 9.5 to 10.3 and the b* value from 19.5 to 21.4 (Table 4.12). The L* value in survey number 2 ranged from 63.2 to 64.7, a* value from 9.5 to 10.1 and the b* value from 18.5 to 19.1. The L* value in survey number 3 ranged from 60.6 to 63.0, a* value from 9.5 to 10.9 and the b* value from 18.4 to 21.4.

The L* value in survey number 4 ranged from 70.4 to 72.4, a* value from 4.3 to 5.0 and the b* value from 11.4 to 12.5. The L* value in survey number 5 ranged from 62.4 to 65.0, a* value from 9.5 to 10.3 and the b* value from 21.3 to 22.7. The L* value in survey number 6 was ranged from 46.2 to 48.4, a* value from 29.0 to 31.42 and the b* value from 10.3 to 12.8. There was no L*, a*, b* differences between survey number 1, survey number 3, and survey number 5 with 2 days and 7 days of storage in the same type of sausage (Table 4.12).

Table 4.12 Color (L*, a*, b*) of chicken sausages after chill storage

Data	**Survey 1**			**Survey 2**			**Survey 3**			**Survey 4**			**Survey 5**			**Survey 6**		
logger no.	L*	a*	b*	L*	a*	b*	L*	a*	b*	L*	a*	b*	L*	a*	b*	L*	a*	b*
1	62.52	10.14	19.56	63.91	9.93	18.62	62.32	9.78	20.31	71.27	4.60	11.46	65.03	9.64	21.31	48.15	29.23	10.34
2	62.50	9.95	19.72	64.03	9.71	18.90	62.46	10.33	20.29	71.67	4.82	11.73	64.46	9.78	21.88	47.32	31.42	11.60
3	62.71	9.94	19.94	64.21	9.90	18.90	62.53	10.58	20.09	71.08	4.84	11.55	62.70	10.01	21.81	47.34	29.86	11.63
4	62.39	9.89	19.57	64.03	9.84	18.61	62.13	10.44	19.58	71.78	4.49	12.38	62.55	9.81	21.78	46.73	30.50	12.54
5	62.45	9.49	19.19	64.70	10.00	19.00	62.60	10.11	18.47	70.96	4.52	11.78	62.71	9.89	21.68	46.93	30.43	11.43
6	61.84	10.22	19.45	63.60	10.04	18.79	62.11	10.39	18.41	71.76	4.38	12.30	62.82	9.93	22.42	46.72	31.10	12.76
7	62.24	10.23	18.72	64.26	10.16	18.98	62.04	10.45	20.43	71.01	4.74	11.35	63.20	10.03	21.85	47.74	30.10	11.29
8	62.48	10.30	20.18	63.55	9.52	18.47	62.19	10.91	19.23	71.26	4.94	12.10	62.61	9.73	21.87	46.95	29.42	11.56
9	62.56	9.57	20.44	64.29	9.97	18.89	63.05	9.63	20.23	70.89	5.01	12.37	63.29	9.94	21.79	47.42	29.36	11.29
10	61.64	10.13	21.19	64.00	9.81	18.98	62.25	10.67	21.11	71.69	4.49	12.16	63.36	9.49	22.56	48.36	30.16	10.29
11	61.56	9.70	21.25	63.41	9.74	18.80	62.23	9.63	21.35	70.43	4.70	11.58	62.99	9.58	22.66	46.58	30.11	11.38
12	61.73	10.04	21.12	64.01	10.09	19.00	62.47	9.83	21.06	71.82	4.48	12.11	64.16	10.02	22.11	46.50	29.75	11.41
13	62.16	9.96	20.87	64.74	9.74	19.06	61.52	9.99	21.18	72.44	4.33	12.31	62.59	9.80	21.65	46.71	28.57	11.34
14	62.41	10.33	21.02	64.19	9.87	18.52	61.42	10.14	21.19	71.67	4.32	11.80	62.92	9.74	21.64	47.47	29.03	11.34
15	63.02	9.98	21.31	63.85	9.83	18.93	61.79	10.19	20.61	72.04	4.46	12.13	62.72	9.91	21.75	47.45	29.74	11.72
16	62.23	10.00	21.36	64.45	9.59	18.89	62.33	9.50	20.81	70.97	4.71	12.50	62.58	9.75	21.65	46.30	29.97	11.43
17	61.62	9.80	21.01	63.98	9.97	18.99	60.97	10.66	20.71	70.94	4.47	11.87	63.35	9.58	21.80	46.58	29.47	11.63
18	61.30	9.86	20.85	63.23	9.70	18.86	60.61	10.05	20.70	70.60	4.63	11.72	62.38	9.61	21.81	46.21	29.88	11.92

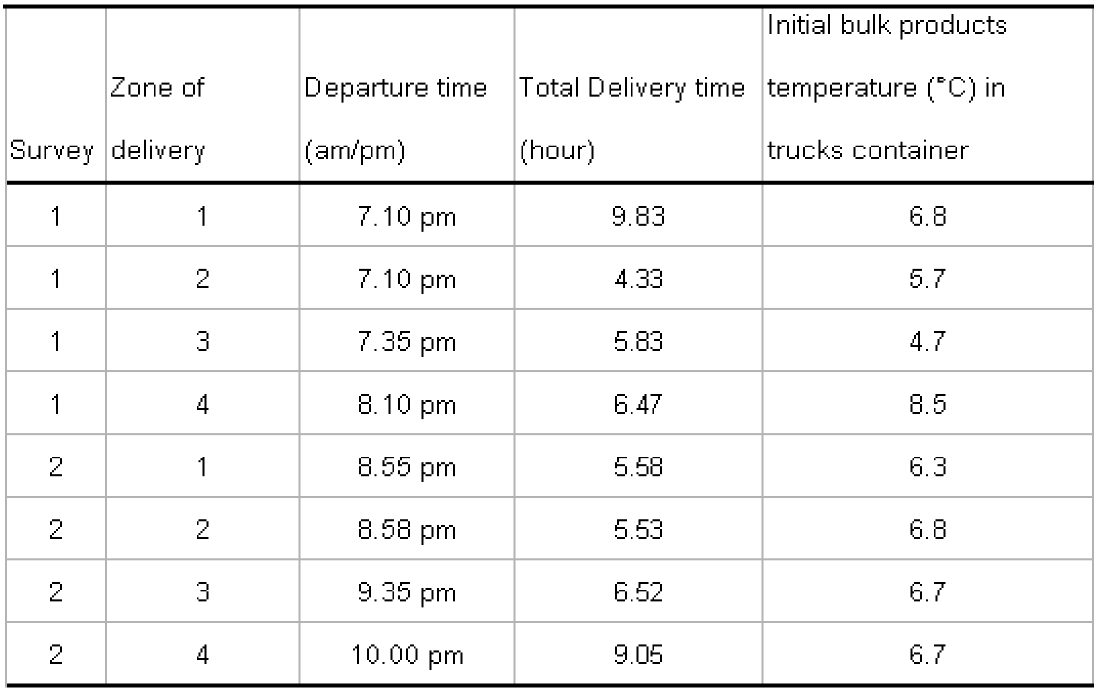
Survey 1, 3 and 5 same type of sausage (corn cheese chicken sausage), survey no. 2 (smoky bite), survey no. 4 (squid sausage) and survey no. 6 (hot dog chicken sausage)

4.2 Temperature survey of chicken sausages during distribution to retailers

4.2.1 Time-temperature profile of chicken sausages during distribution

The real temperature history of chicken sausages was monitored during distribution to retailers around Bangkok area. Delivery area was divided into four zones with four unrefrigerated pick-up trucks. In each truck (5.148 m^3) which was fully loaded contained five of sausage packages equipped with data logger in order to record the temperature history during delivery period. In this survey, the initial temperature of the products was taken using a thermocouple. The initial temperature ranged from 4.7 to 8.5°C in the first survey and 6.3 to 6.8°C in the second survey. Departure and delivery time were recorded in each survey (Table 4.13).

Table 4.13 Time and temperature of chicken sausages before distribution with different zone in Bangkok area



Survey	Zone of delivery	Departure time (am/pm)	Total Delivery time (hour)	Initial bulk products temperature (°C) in trucks container
1	1	7.10 pm	9.83	6.8
1	2	7.10 pm	4.33	5.7
1	3	7.35 pm	5.83	4.7
1	4	8.10 pm	6.47	8.5
2	1	8.55 pm	5.58	6.3
2	2	8.58 pm	5.53	6.8
2	3	9.35 pm	6.52	6.7
2	4	10.00 pm	9.05	6.7

Delivery time of the sausages started in the evening from 7.10pm to 10.00pm and ended between 11.43 pm until 7.03 am on the next morning. All trucks delivered chill sausages around fifty places surrounding Bangkok area. The result showed that, the lowest temperature was found mostly in the middle part of truck container. The longest delivery time took place in zone 1 in the first survey and zone 4 in the second survey (Table 4.13). Higher temperatures appeared mostly in the lower level at the back side of truck container (Figure 4.9 to figure 4.16). The sausages located at back lower level (BL) took the longest delivery time of 9.05 hours in survey number 2 out of four zones of delivery (Table 4.15 and Figure 4.16). Similar result was also found in the sausages delivered in zone 2, zone 3 and zone 4 for survey number 1 while in zone 1 and zone 4 for survey number 2 (Figure 4.9 to figure 4.16).

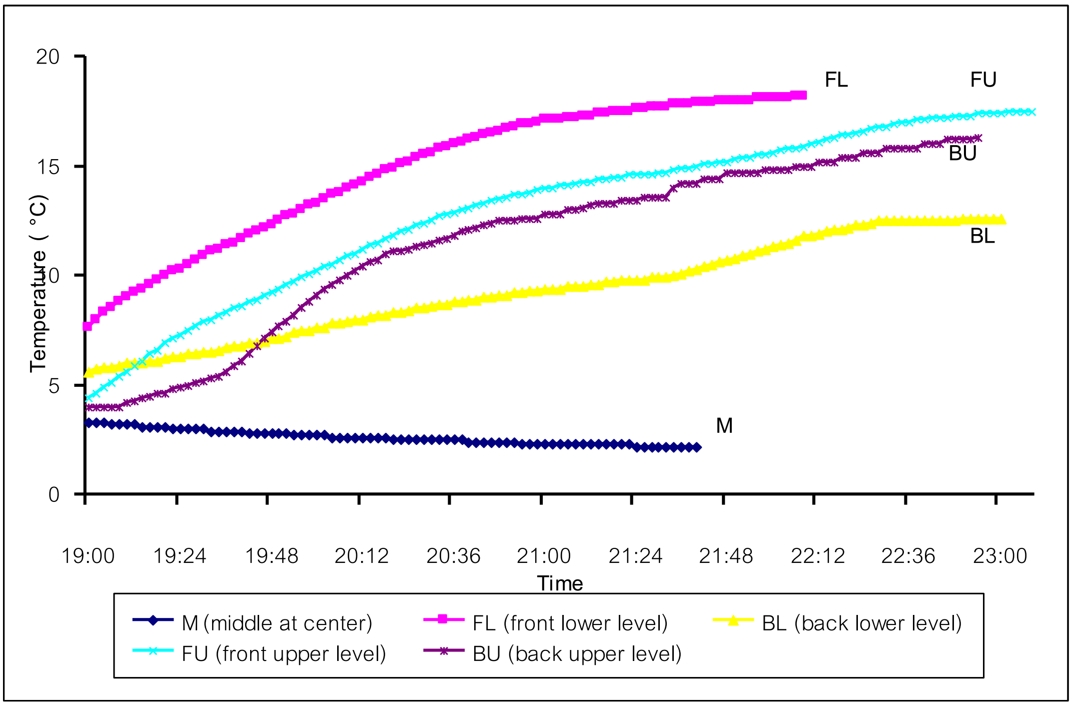


Figure 4.9 Temperature profile of chicken sausages located at different places in the delivery truck during distribution to zone 1 in survey number 1

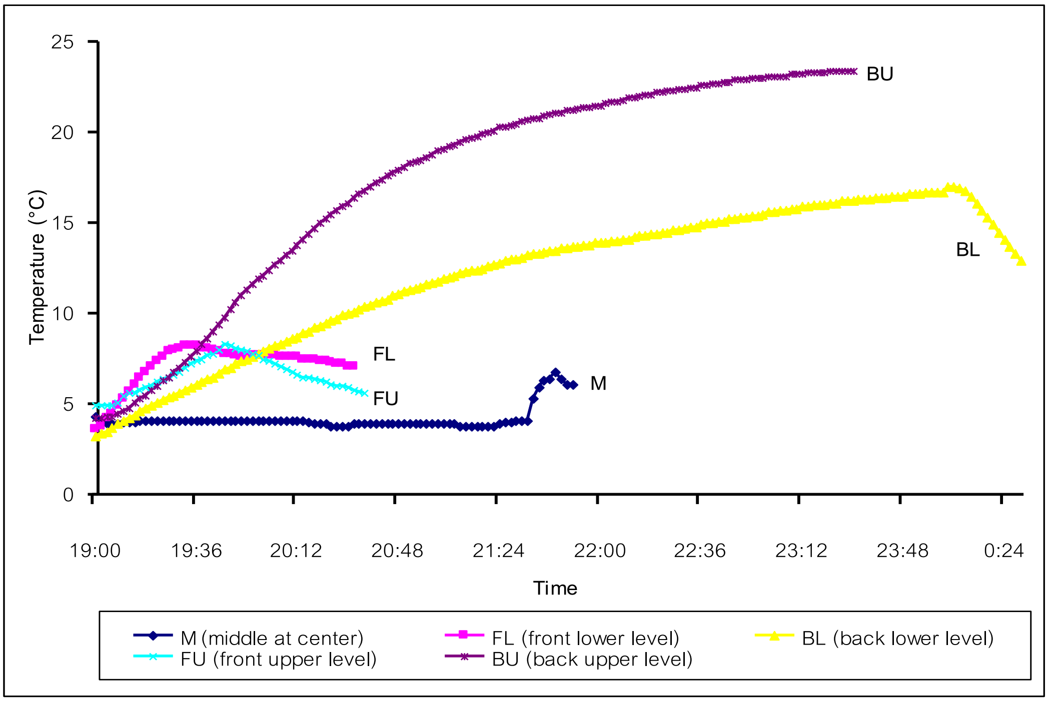


Figure 4.10 Temperature profile of chicken sausages located at different places in the delivery truck during distribution to zone 2 in survey number 1.

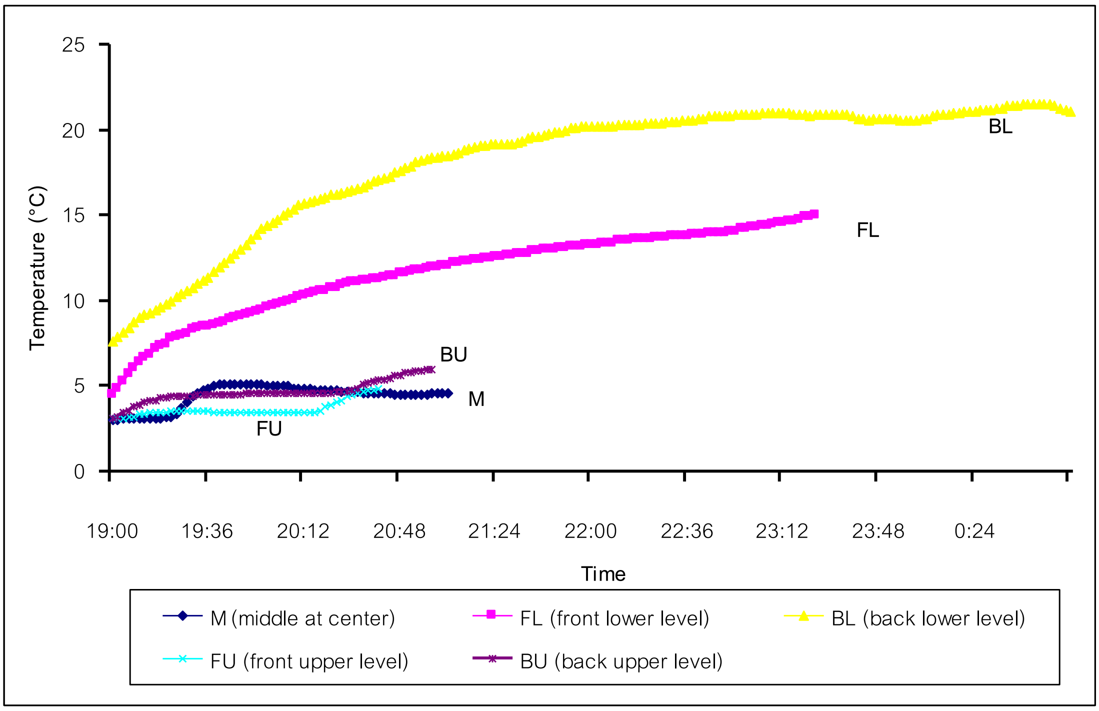


Figure 4.11 Temperature profile of chicken sausages located at different places in the delivery truck during distribution to zone 3 in survey number 1.

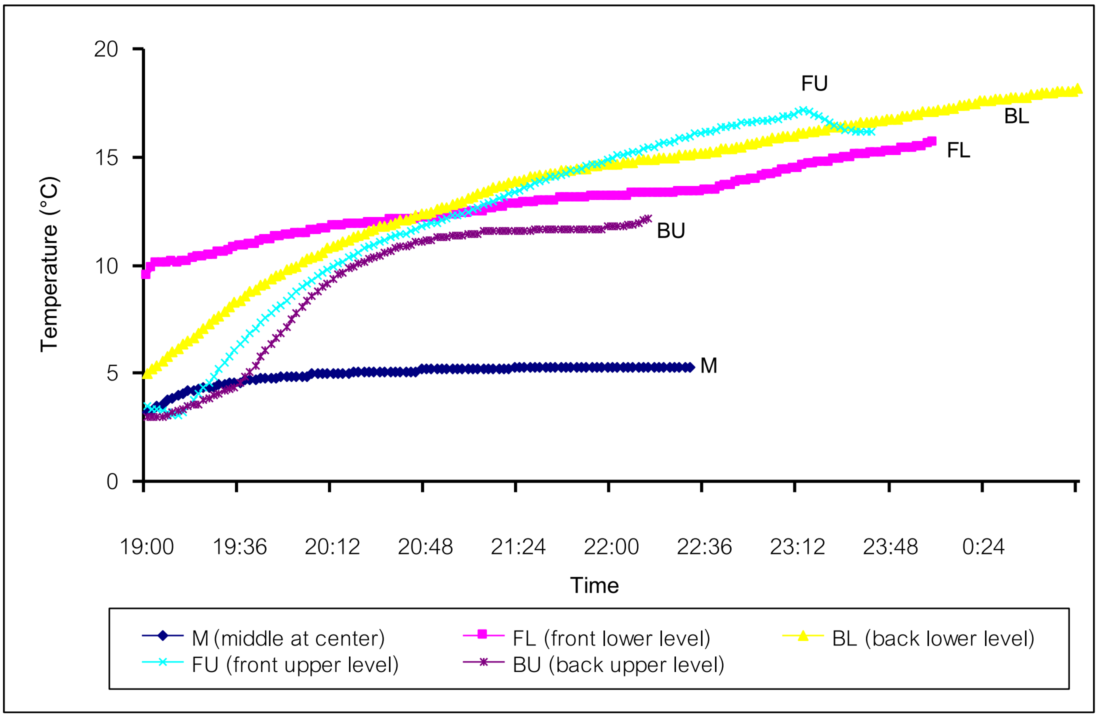


Figure 4.12 Temperature profile of chicken sausages located at different places in the delivery truck during distribution to zone 4 in survey number 1.

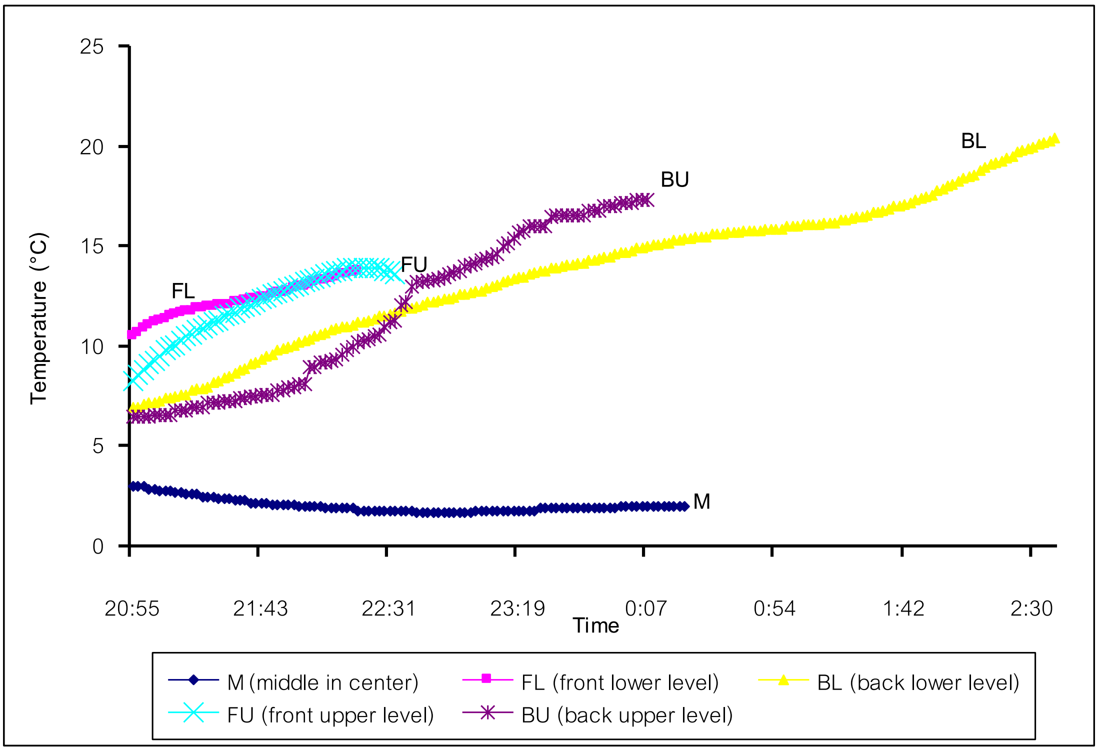


Figure 4.13 Temperature profile of chicken sausages located at different places in the delivery truck during distribution to zone 1 in survey number 2

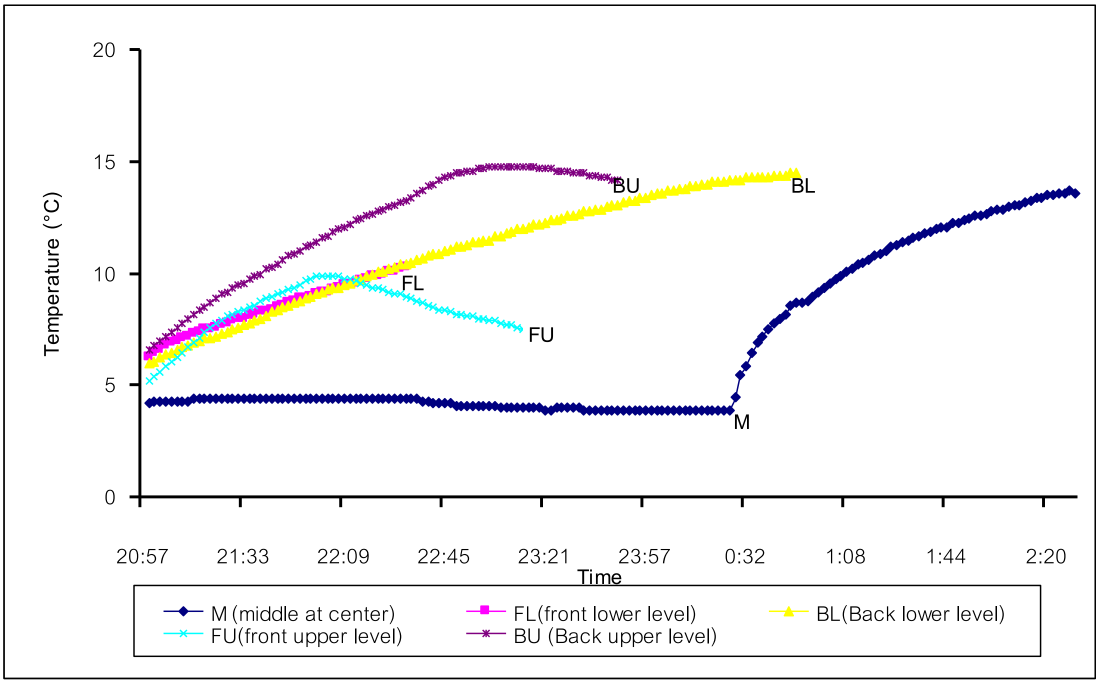


Figure 4.14 Temperature profile of chicken sausages located at different places in the delivery truck during distribution to zone 2 in survey number 2

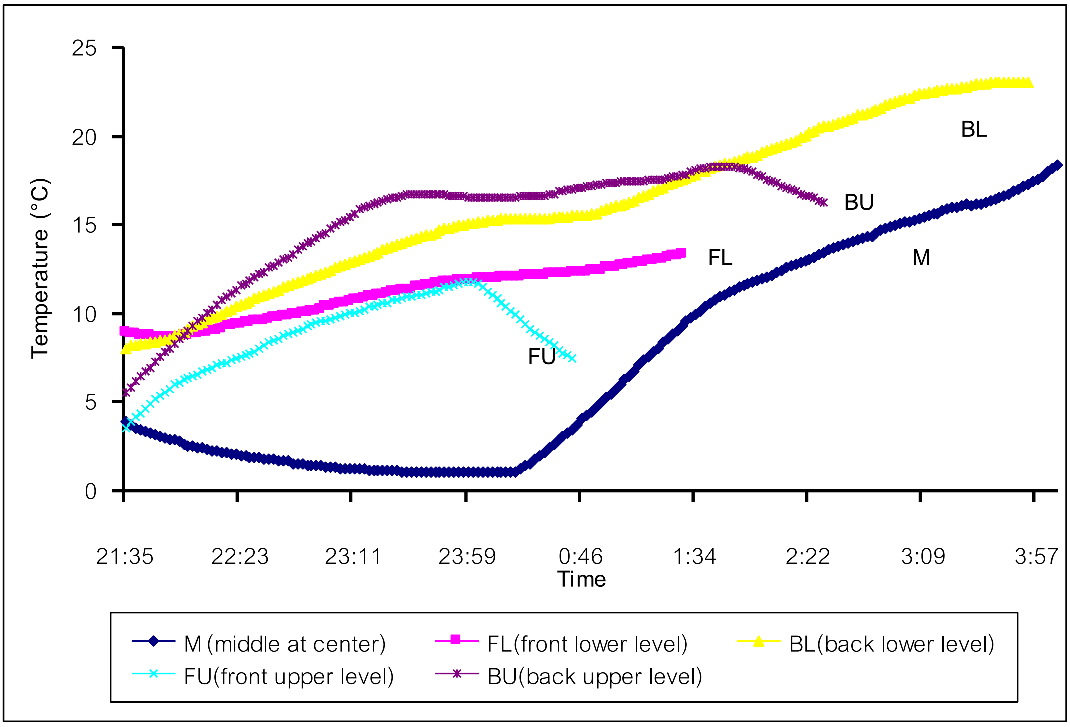


Figure 4.15 Temperature profile of chicken sausages located at different places in the delivery truck during distribution to zone 3 in survey number 2

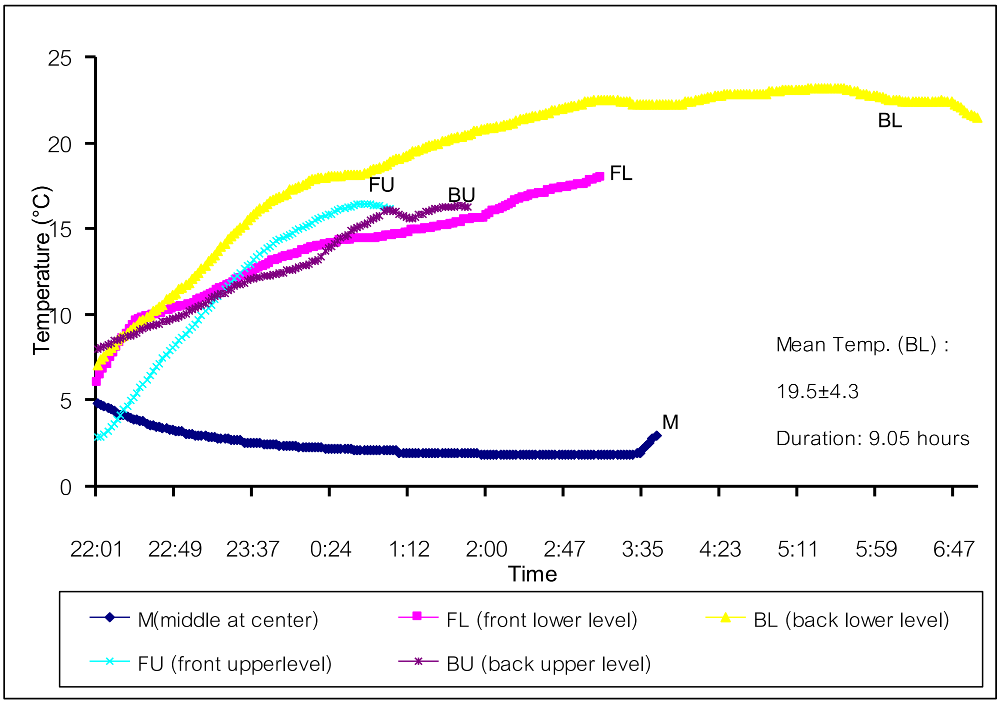


Figure 4.16 Temperature profile of chicken sausages located at different places in the delivery truck during distribution to zone 4 in survey number 2

The result showed that, the temperature difference range was between 1.1°C to 19.2°C in both surveys (Table 4.14 and Table 4.15). It was found that, in five locations out of eight data set from two surveys in four zones, the majority higher temperature rise were likely found in the sausages located at back lower level (BL) where outside warm air came in contact more easily and mostly associated with longer delivery time (Table 4.14 and Table 4.15). This was because most of the delivery time ranged from 4 to 9 hour. Longer the delivery period caused the temperature rise during distribution. From the result the sausages located at back lower level (BL) experienced the highest temperature rise ranged 7°C to 16.1°C (Table 4.14 and Table 4.15).

Table 4.14 Temperature survey result data of chicken sausages during distribution in a truck container to four zones in survey number 1

Location of data logger	**Zone**	**Max temp °C**	**Min temp°C**	**Temperature difference °C**	**Transportation duration (hour)**
M:middle at center	1	3.3	2.2	1.1	2.33
FL :front lower level	1	18.2	7.6	10.6	2.83
BL:back lower level	1	12.6	5.6	7.0	3.83
FU: front upper level	1	17.5	4.4	13.1	4.00
BU:back upper level	1	16.3	4.0	12.3	9.83
M:middle at center	2	6.8	3.8	3.0	2.58
FL :front lower level	2	8.2	3.6	4.6	0.33
BL:back lower level	2	17.0	3.2	13.8	4.33
FU: front upper level	2	8.3	4.9	3.4	0.67
BU:back upper level	2	23.4	4.2	19.2	3.33
M:middle at center	3	5.1	3.0	2.1	1.42
FL :front lower level	3	15.0	4.5	10.5	4.33
BL:back lower level	3	21.5	7.6	13.9	5.83
FU: front upper level	3	4.9	3.1	1.8	1.50
BU:back upper level	3	6.0	3.1	2.9	1.67
M:middle at center	4	5.3	3.2	2.1	3.28
FL :front lower level	4	15.7	9.5	6.2	4.07
BL:back lower level	4	19.4	5.0	14.4	6.47
FU: front upper level	4	17.2	3.1	14.1	4.37
BU:back upper level	4	12.2	3.0	9.2	3.08

Temperature highlighted in red color font was the highest temperature, highest temperature difference and longest time and font in blue color box was the lowest temperature, lowest temperature difference and the shortest time in each locations and zones

Table 4.15 Temperature survey result data of chicken sausages during distribution in a truck container to four zones in survey number 2

Location of data logger	**Zone**	**Max temp °C**	**Min temp°C**	**Temperature difference °C**	**Transportation duration (hour)**
M:middle at center	1	3.0	1.7	1.3	3.42
FL :front lower level	1	13.8	10.5	3.3	1.25
BL:back lower level	1	20.4	7.0	13.4	5.58
FU: front upper level	1	13.9	8.3	5.6	1.33
BU:back upper level	1	17.3	6.5	10.8	5.58
M:middle at center	2	13.7	3.9	9.8	5.53
FL :front lower level	2	10.3	6.3	4.0	1.53
BL:back lower level	2	14.5	6.0	8.5	3.87
FU: front upper level	2	9.9	5.2	4.7	2.03
BU:back upper level	2	14.8	6.6	8.2	1.53
M:middle at center	3	18.4	1.1	17.3	6.52
FL :front lower level	3	13.4	8.7	4.7	3.92
BL:back lower level	3	23.1	8.1	15.0	6.00
FU: front upper level	3	11.8	3.6	8.2	3.01
BU:back upper level	3	18.3	5.6	12.7	4.92
M:middle at center	4	4.9	1.9	3.0	5.72
FL :front lower level	4	18.3	5.6	12.7	5.17
BL:back lower level	4	23.2	7.1	16.1	9.05
FU: front upper level	4	16.5	2.9	13.6	3.00
BU:back upper level	4	16.4	8.0	8.4	3.78

Temperature highlighted in red color font was the highest temperature, highest temperature difference and longest time and font in blue color box was the lowest temperature, lowest temperature difference and the shortest time in each locations and zones

The results showed that, the sausages that were placed at the center in the middle of a truck container was low in temperature compared with other locations. The bulks of chilled sausages were more compact and crowded in the center and that is the reason. The sausages placed in the middle of the container stayed in appropriate chill temperature due to less of heat and air circulation of ambient temperature in truck container. Therefore, the products maintained as prescribed in ATP Agreement for chill temperature meat products (0° to 5°C) during distribution to retailer. According to the Agreement on the International Carriage of Perishable Foodstuffs (ATP Agreement), the maximum temperature for distribution of poultry products should be lower than or at least 4°C. The result also showed that, most of the temperatures exceeded 10°C with more than three hours delivery time.

In the survey of James et al. (2008), it is accepted if the temperature does not exceed 10°C for more than three hours (Figure 4.9 to 4.16). Higher temperatures were found mostly for sausages that were placed at back lower level (BL) of truck container. In this study, by delivering the sausages in the evening helped to slow down the temperature increase in the products during distribution. Nonetheless, the temperature rise was still found to be as high as 19.2 °C .To minimize this problem, the sausages should be delivered within 3 hours. This survey showed that, when the sausages initial temperature was lower than 5°C and they were protected from outside heat during transportation, delivering time could be extended to 3 to 4 hours before the final temperature reached above 5°C (Table 4.14 and Table 4.15).

The chance of spoilage and growth of food poisoning bacteria could be minimize if the sausages were outside the temperature danger zone (> 5°C). The result showed that, 50% of the surveyed sausages had the temperature above the specific limits (0 to 5°C) of chill food products during distribution.

4.2.2 Microbial and physico-chemical analysis of chicken sausages after distribution to retailer

4.2.2.1 Microbiology analysis of chicken sausages after distribution

The results showed that TPC of the sausages was high and mostly (57.5%) exceeded the maximum limit of TPC (10^5 cfu/g) that was allowed by the Thai Industrial Standard of sausages (TISI). The frequency of TPC exceeding the limit was 23 out of 40 samples from the survey. The highest TPC was 7.12 log of cfu/g located at the back lower level (BL) of truck container delivered in zone 3 for survey number 1. The lowest TPC was 4.14 log of cfu/g (FL) located at front lower level of truck container delivered in zone 2 for survey number 1. The highest TPC for survey number 2 was 7.38 log of cfu/g located at back upper level (BU) of truck container delivered in zone 3. The lowest TPC was 4.05 log of cfu/g located at front upper level (FU) of truck container delivered in zone 1 for survey number 2 (Table 4.16). The results also showed that, most of the mean TPC were likely low in the sausages in the middle (M) of truck container (Table 4.16).

Table 4.16 Total plate count of chicken sausages after distribution with different locations in truck container and different zone of delivery from two surveys

Location of data logger	**Zone**	**Survey 1**		**Survey 2**	
		(log cfu/g)	Delivery time (hour)	(log cfu/g)	Delivery time (hour)
M:middle at center	1	4.82	2.33	5.05	3.42
FL :front lower level	1	5.11	2.83	7.11	1.25
BL:back lower level	1	5.71	3.83	6.70	5.58
FU: front upper level	1	5.60	4.00	4.05	1.33
BU:back upper level	1	4.81	9.83	4.59	5.58
M:middle at center	2	4.48	2.58	4.62	5.53
FL :front lower level	2	4.14	0.33	4.90	1.53
BL:back lower level	2	4.71	4.33	4.63	3.87
FU: front upper level	2	3.86	0.67	4.76	2.03
BU:back upper level	2	4.81	3.33	4.57	1.53
M:middle at center	3	5.52	1.42	7.24	6.52
FL :front lower level	3	6.13	4.33	6.97	3.92
BL:back lower level	3	**7.12**	5.83	7.21	6.00
FU: front upper level	3	4.75	1.50	6.99	3.01
BU:back upper level	3	5.70	1.67	**7.38**	4.92
M:middle at center	4	5.92	3.28	4.09	5.72
FL :front lower level	4	6.79	4.07	5.11	5.17
BL:back lower level	4	7.06	6.47	5.71	9.05
FU: front upper level	4	6.49	4.37	4.17	3.00
BU:back upper level	4	6.27	3.08	5.51	3.78

The red color font indicated the TPC>5 log of cfu/g

There was a relationship between delivery time and TPC in sausages stored at different locations in a truck container during distribution. Higher TPC was most likely associated with longer delivery time. The results also showed that, the sausages distributed for more than 6 hours was most likely higher in TPC after distribution (Table 4.16). It was shown that, higher count of TPC related with higher temperature which was associated in certain locations in a truck container. The survey data indicated that, the lower level of truck container experienced higher temperature rise and thus resulted in higher load in TPC after distribution (Table 4.14, Table 4.15 and Table 4.16).

Some of the sausages spoiled after distribution due to high temperature with longer delivery time. Foster (2007) reported that an increase in average temperature significantly decreases the length of retention and quality of many chilled foods and subsequently leads to decrease in safety and shelf life of the food product. High temperatures could lead to increasing the growth of several spoilage microorganisms during storage and distribution of chicken products (Pothakos et al., 2012).

4.2.3 Physico-chemical analysis of chicken sausages after distribution

4.2.3.1 pH and Titratable acidity

The result showed that, pH value ranged from 5.71 to 6.46 for survey number 1 and from 6 to 6.4 for survey number 2 (Table 4.17). The initial pH of the sausages after cooking was 6.5 and after peeling off the casing was 6.4. After the distribution pH slightly decreased and the acidity of the sausages were slightly increased. The lowest pH was found at back lower level (BL) on truck container delivered in zone 3 for survey number 1 and at middle (M) of truck container in the same zone for survey number 2 (Table 4.17). The result showed that the TA ranged from 0.043% to 0.071% after distribution in survey number 1 and from 0.047% to 0.069% in survey number 2 (Table 4.17). The result showed that higher TA associated with lower pH. The highest TA was found at back lower level (BL) in zone 3 for survey number 1 and at middle (M) of the truck delivering the products in the same zone for survey number 2 (Table 4.17). The initial TA of the sausage after cooked was 0.042%. The result shows that the TA value of the sausages increased after distribution. The results also showed that, there was a relationship between pH and TA 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.

Table 4.17 pH and % of titratable acidity (TA) of chicken sausages after distribution in different locations in a truck delivering the products in different zones from two surveys

Location of data logger	**Zone**	**Survey 1**		**Survey 2**	
		pH	%TA	pH	%TA
M:middle at center	1	6.45	0.048	6.41	0.054
FL :front lower level	1	6.31	0.054	6.29	0.063
BL:back lower level	1	6.32	0.054	6.50	0.047
FU: front upper level	1	6.31	0.053	6.52	0.047
BU:back upper level	1	6.24	0.059	6.42	0.054
M:middle at center	2	6.25	0.056	6.44	0.051
FL :front lower level	2	6.48	0.043	6.41	0.053
BL:back lower level	2	6.38	0.053	6.41	0.056
FU: front upper level	2	6.36	0.053	6.39	0.060
BU:back upper level	2	6.50	0.045	6.41	0.057
M:middle at center	3	6.25	0.053	5.99	0.069
FL :front lower level	3	6.46	0.048	6.31	0.059
BL:back lower level	3	5.69	0.084	6.38	0.059
FU: front upper level	3	6.32	0.056	6.39	0.054
BU:back upper level	3	6.24	0.062	6.50	0.047
M:middle at center	4	6.28	0.056	6.35	0.056
FL :front lower level	4	6.26	0.059	6.41	0.050
BL:back lower level	4	5.96	0.071	6.42	0.057
FU: front upper level	4	5.71	0.071	6.31	0.063
BU:back upper level	4	6.30	0.059	6.49	0.051

The red font highlighted the lowest pH and the highest titratable acidity (TA) in each locations and zones, TA unit: % acidity

4.2.3.2 Texture

The texture result showed that the shear force after distribution was in between 6.9 N to 8.7 N for survey number 1 and from 7.4N to 9.9N for survey number 2 in different locations of truck container with different zone of delivery (Table 4.18). The shear force of the sausages before distribution ranged from 7.6N to 11.1N and after distribution, the shear force reduced to 6.9N to 9.9N. The highest shear force was observed in the sausages located at the middle (M) of truck container that delivered to zone 1 in survey number 1 and front upper level (FU) of truck container that delivered in zone 2 of survey number 2 (Table 4.18). The lowest shear force was found at front upper level (FU) in survey number 1 (zone 1) and same location for survey number 2 but in zone 3 (Table 4.18).The result also showed that, there was a relationship associating shear force with temperature of the sausage. Lower the temperature difference during distribution caused the shear force of the sausages to be higher (Table 4.14, Table 4.15 and Table 4.18).

Table 4.18 Texture of chicken sausages after distribution in different locations in a truck delivering the products in different zones from two surveys

Location of data logger	**Zone**	**Survey 1**	**Survey 2**
		Shear force (N)	
M:middle at center	1	8.7	9.4
FL :front lower level	1	7.4	8.6
BL:back lower level	1	7.1	9.6
FU: front upper level	1	6.9	9.0
BU:back upper level	1	7.1	9.3
M:middle at center	2	7.4	8.6
FL :front lower level	2	7.1	8.7
BL:back lower level	2	7.0	9.7
FU: front upper level	2	7.1	9.9
BU:back upper level	2	8.0	9.5
M:middle at center	3	7.6	8.8
FL :front lower level	3	7.2	9.0
BL:back lower level	3	7.2	9.2
FU: front upper level	3	8.3	7.4
BU:back upper level	3	7.9	8.8
M:middle at center	4	7.8	9.1
FL :front lower level	4	8.4	9.5
BL:back lower level	4	7.1	9.2
FU: front upper level	4	7.2	9.3
BU:back upper level	4	7.1	9.5

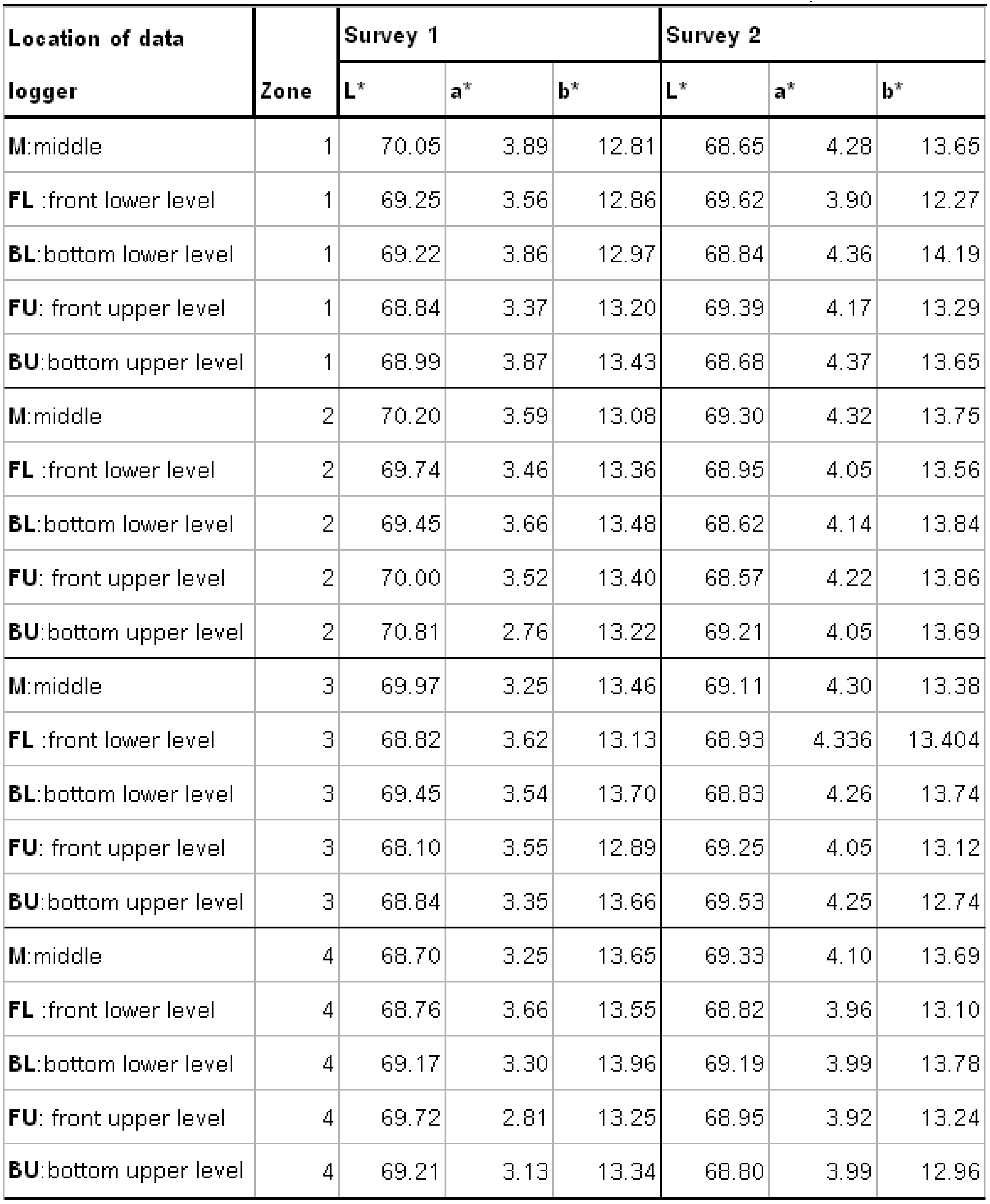
The red highlighted box indicated the lowest shear force while the blue highlighted box indicated the highest shear force in each survey

Thomas et al. (2008) reported that, shear force of the pork sausages were decreased significantly with decrease in pH due to protein denaturation increased and subsequent loss of binding properties of meat protein. The result from this survey showed inconsistency relationship associating shear force value and pH. For example in survey number 1 where the lowest shear force located at FU in zone 1 but the lowest pH located at BL in zone 3. The similar result observed in survey number 2 where the lowest shear force located at FU in zone 3 while the lowest pH was located at M in the same zone (Table 4.17 and Table 4.18).

4.2.3.3 Color (L*, a*, b*)

The result showed that the lightness (L*) of the squid chicken sausages from those two surveys after distribution ranged from 69 to 70, redness (a*) from 2.8 to 4.4 and yellowness (b*) value from 12.3 to 14.0 (Table 4.19). Color results showed that this type of sausage was pale white in color. The (L*, a*, b*) color values did not vary in different locations in the truck after distribution. The color of the fresh squid sausage was L*= 70.82, a*= 4.67 and b*= 13.52. The result also showed that there was no difference in color between two surveys after the delivery to different zones of distribution (Table 4.19). L*, a*, b* values of the sausages did not related with temperature difference and delivery period after distribution period in two surveys with four different zones.

Table 4.19 Color (L*, a*, b*) value of chicken sausages after distribution on a truck container to four zones of delivery in two surveys



Location of data logger	**Zone**	**Survey 1**			**Survey 2**		
		L*	a*	b*	L*	a*	b*
M:middle	1	70.05	3.89	12.81	68.65	4.28	13.65
FL :front lower level	1	69.25	3.56	12.86	69.62	3.90	12.27
BL:bottom lower level	1	69.22	3.86	12.97	68.84	4.36	14.19
FU: front upper level	1	68.84	3.37	13.20	69.39	4.17	13.29
BU:bottom upper level	1	68.99	3.87	13.43	68.68	4.37	13.65
M:middle	2	70.20	3.59	13.08	69.30	4.32	13.75
FL :front lower level	2	69.74	3.46	13.36	68.95	4.05	13.56
BL:bottom lower level	2	69.45	3.66	13.48	68.62	4.14	13.84
FU: front upper level	2	70.00	3.52	13.40	68.57	4.22	13.86
BU:bottom upper level	2	70.81	2.76	13.22	69.21	4.05	13.69
M:middle	3	69.97	3.25	13.46	69.11	4.30	13.38
FL :front lower level	3	68.82	3.62	13.13	68.93	4.336	13.404
BL:bottom lower level	3	69.45	3.54	13.70	68.83	4.26	13.74
FU: front upper level	3	68.10	3.55	12.89	69.25	4.05	13.12
BU:bottom upper level	3	68.84	3.35	13.66	69.53	4.25	12.74
M:middle	4	68.70	3.25	13.65	69.33	4.10	13.69
FL :front lower level	4	68.76	3.66	13.55	68.82	3.96	13.10
BL:bottom lower level	4	69.17	3.30	13.96	69.19	3.99	13.78
FU: front upper level	4	69.72	2.81	13.25	68.95	3.92	13.24
BU:bottom upper level	4	69.21	3.13	13.34	68.80	3.99	12.96

Type of sausage in survey number 1 and survey number 2: Squid chicken sausages

4.3 Prediction on microbial quality of chicken sausages during storage and Distribution

4.3.1 Prediction of microbial quality of chicken sausages during storage

The worst case temperature profile data from chilled storage was selected as the sample to predict microorganism growth during chilled storage. All the parameters were collected and the predictive analysis was conducted using the ComBase® microbial predictive software to predict the pathogen growth during the storage. The ComBase® predictor is a tool for predicting the response of pathogens and spoilage microorganisms based on the key environmental factors like temperature, pH and a_w. The model was used to predict the growth of food borne pathogens as a function of the parameter that has been selected. Four pathogens, which were *Pseudomonas spp.*, *Staphylococcus aureus*, *Salmonella* spp. and *Listeria monocytogenes* were selected for growth simulation during storage (Table 4.20). Five parameters were selected to predict the growth of selected pathogens during chilled storage (Table 4.20).

Table 4.20 Pathogen prediction parameter, rate and doubling time during chill storage

Type of Pathogen	**Maximum temperature (°C)**	**Observation period (hours)**	**pH**	**Water activity (a_W)**	**Initial level log (cfu/g)**	**Max rate (log.conc./h)**	**Double time (hours)**
Staphylococcus aureus	8.4	6.3	6.83	0.98	0.1	0.014	21.92
Salmonella spp.	8.4	6.3	6.83	0.98	0.1	0.015	19.56
Listeria monocytogenes	8.4	6.3	6.83	0.98	0.1	0.034	8.84
Pseudomonass spp.	8.4	6.3	6.83	0.98	0.1	0.061	4.97

The growth simulation for *Escherichia coli and Clostridium perfringens* could not be performed using the ComBase® software because the simulation software did not allow the growth simulation from 10°C to 42°C for *E.coli* and for *Clostridium perfringens* from 15°C to 52°C.

4.3.1.1 *Pseudomonas* spp. prediction

The result showed that, *Pseudomonas* spp grew rapidly after three hours during chill storage at 8.4°C. The lag phase of *Pseudomonas* was less than an hour before it grew constantly relatively rapid after this hour (Figure 4.17). The rate of *Pseudomonas* spp growth was at 0.061 log concentration per hour. The doubling time of the *Pseudomonas* spp was 4.97 hours, which means the *Pseudomonas* spp was able to double the numbers in every 4 hour 58 minutes and 2 second during storage (Table 4.20). *Pseudomonas* spp. reached the 0.2 log of CFU/g after 6 hours of storage when the initial count was 0.1 log CFU/g.

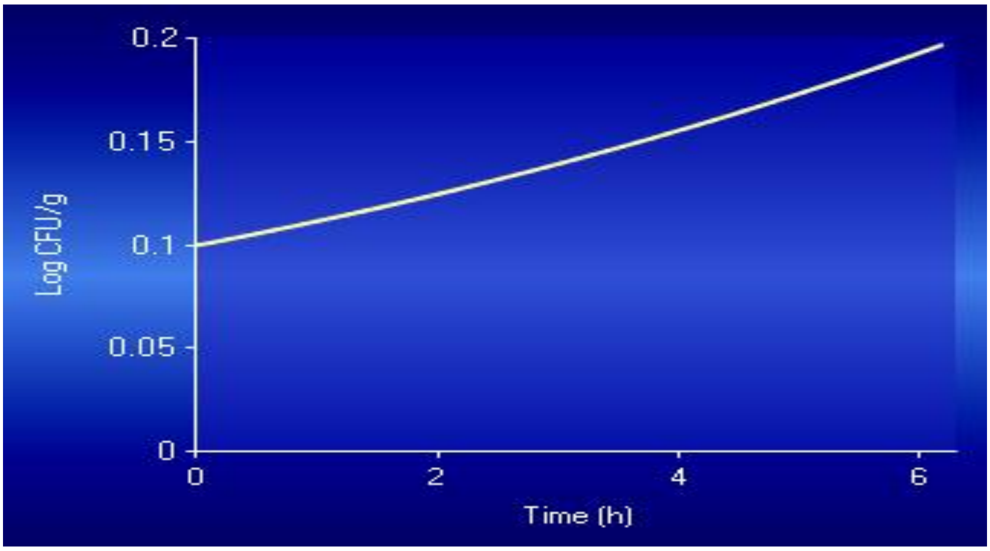


Figure 4.17 *Pseudomonas* spp. growth prediction during storage at 8.4°C, pH:6.83, a_w :0.98 and for 6.3 hours observation period.

4.3.1.2 *Staphylococcus aureus* prediction

The result showed that *Staphylococcus aureus* grew slowly after four hours during chill storage at 8.4°C. The growth rate of *Staphylococcus aureus* was at 0.014 log concentration per hour. The initial Staphylococcus *aureus* was estimated at 0.1 log CFU/g and grew at 0.014 log concentration in every hour of chill storage. The doubling time of the *Staphylococcus aureus* was 21.92 hours, which means *Staphylococcus aureus* was able to double in every 21 hour 55 minutes 2 second during storage (Table 4.20). *Staphylococcus aureus* reached the 0.11 log of CFU/g after 6 hours of storage when the initial count was 0.1 log CFU/g (Figure 4.18).

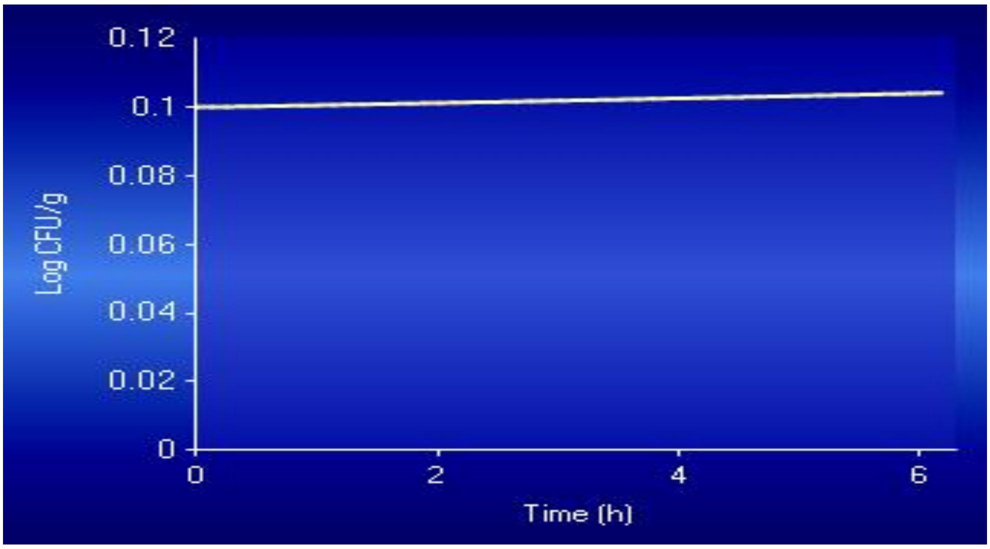


Figure 4.18 *Staphylococcus aureus* growth prediction during storage at 8.4°C, pH:6.83, a_w:0.98 and for 6.3 hours observation period

4.3.1.3 *Salmonella* spp. prediction

The result showed that *Salmonella* spp. grew slowly after four hours during chill storage at 8.4°C. The growth rate of *Salmonella* spp. was at 0.015 log concentration per hour. The doubling time of the *Salmonella* spp. was at 19.56 hours which means *Salmonella* spp. was able to double every 19 hour 33 minutes 6 second during storage at mean temperature 8.4°C (Table 4.20). The *Salmonella* spp. reached 0.11 log CFU/g after 6.3 hours of storage when the initial count was 0.1 log CFU/g (Figure 4.19).

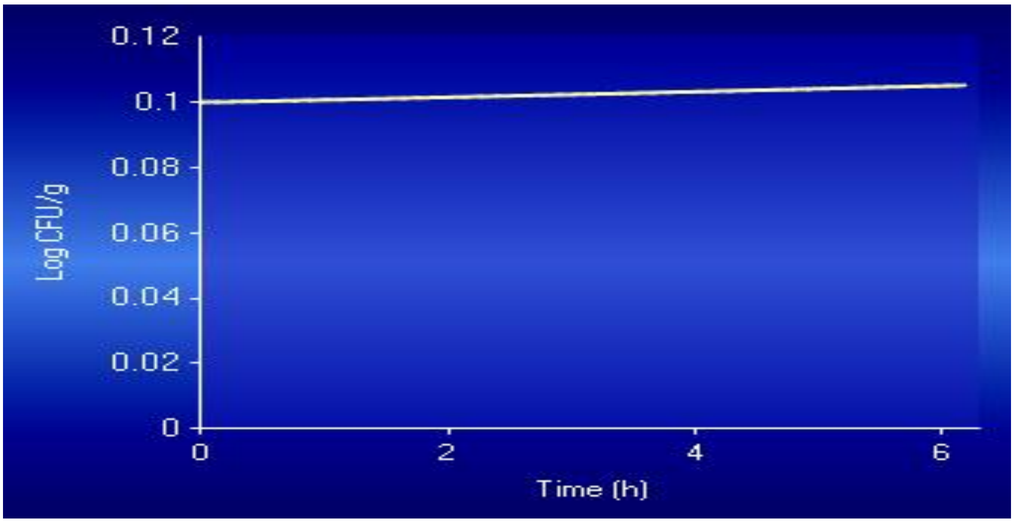


Figure 4.19 *Salmonella* spp growth prediction during storage at 8.4°C, pH:6.83, a_w:0.98 and for 6.3 hours observation period

4.3.1.4 *Listeria monocytogenes* prediction

The result showed that *Listeria monocytogenes* grew slowly after four hours during chill storage at 8.4°C. The growth rate of *Listeria monocytogenes* was 0.034 log concentration per hour. The doubling time of the *Listeria monocytogenes* was 8.84 hours, which means *Listeria monocytogenes* was able to double every 8 hour 50 minutes 20 seconds during storage (Table 4.20). *Listeria monocytogenes* reached 0.11

log CFU/g after 6 hours of storage when the initial count was 0.1 log CFU/g (Figure 4.20).

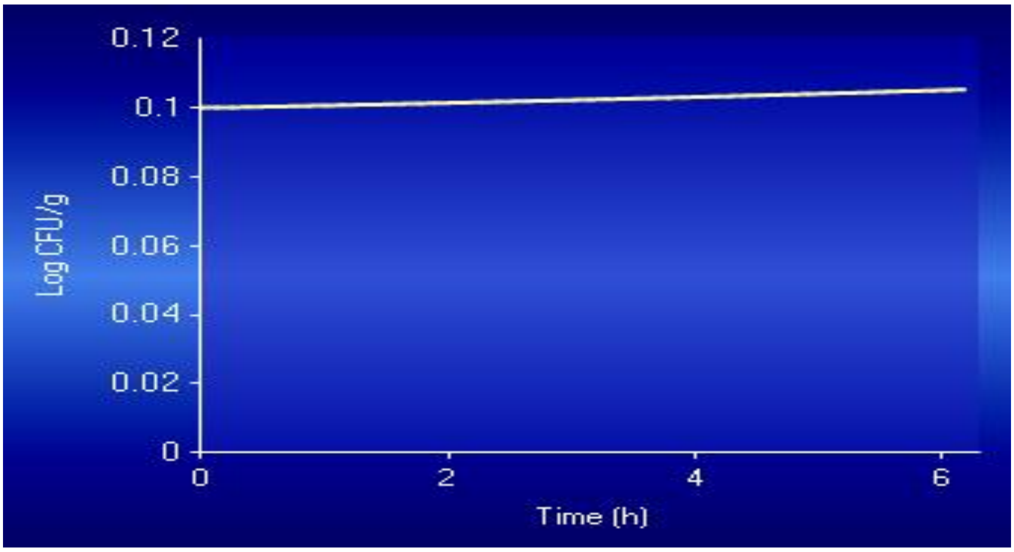
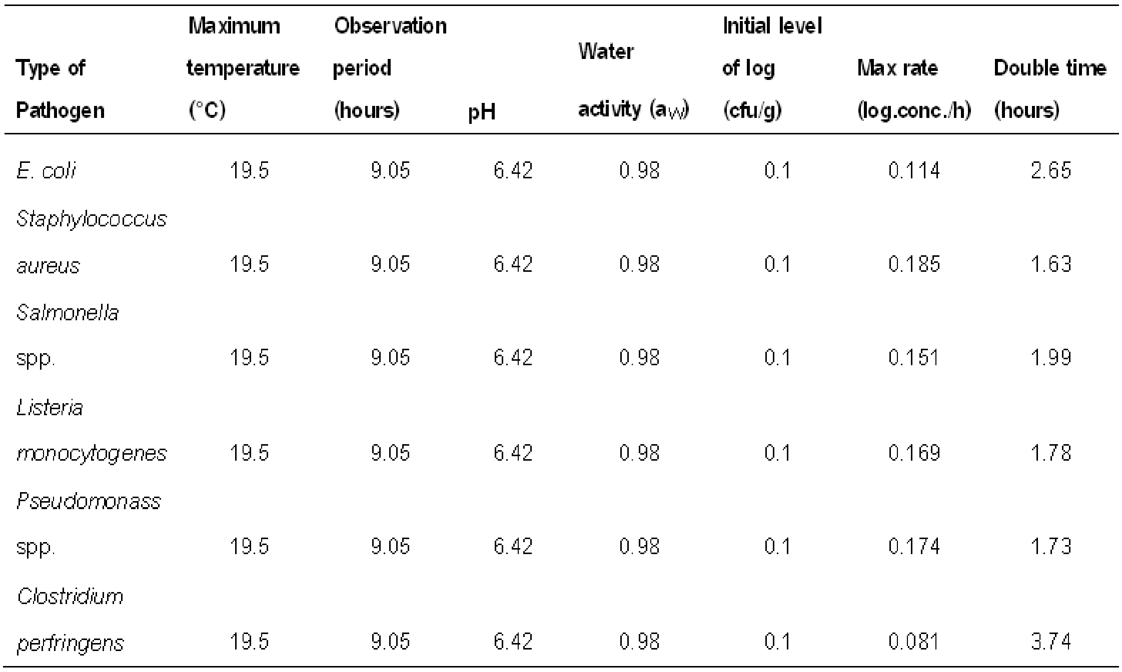


Figure 4.20 *Listeria monocytogenes* growth prediction during storage at 8.4°C, pH:6.83, a_w:0.98 and for 6.3 hours observation period

4.3.2 Prediction on microbial quality of chicken sausages during distribution to Retailer

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 storage (Table 4.21). Five parameters were selected to predict the growth of selected pathogens during chilled storage as listed in Table 4.21.

Table 4.21 Pathogen prediction parameter, rate and doubling time during distribution or delivery to retailer



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

4.3.2.1 *Salmonella* spp. prediction

The result showed that, *Salmonella* spp. grew rapidly after 3 hours during distribution at 19.5°C (Table 4.21). The growth rate of *Salmonella* was 0.151 log concentration per hour. The doubling time of *Salmonella* spp. was 1.99 hours, which means Salmonella spp. was able to double in every 2 hour during distribution (Table 4.21). *Salmonella* spp. reached 0.4 log of CFU/g after 9.05 hours during distribution time when the initial count was 0.1 log CFU/g. (Figure 4.21).

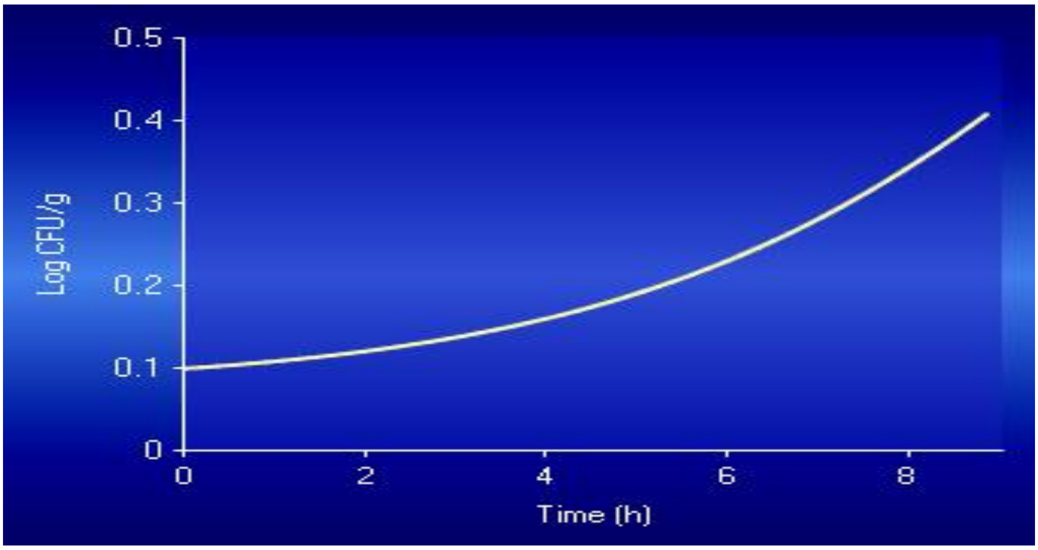


Figure 4.21 Growth prediction of *Salmonella spp*. during distribution at 19.5°C, pH:6.42, a_w:0.98 and for 9.05 hours observation period

4.3.2.2 *Escherichia coli* prediction

The result showed that *E.co*li grew rapidly after 4 hours during distribution at 19.5°C. The growth rate of *E.coli* was at 0.114 log concentration per hour. The doubling time for *E.coli* is 2.65 hours, which means *E.coli* was able to double in every 2 hour and 39 minutes during distribution (Table 4.21). *E.coli* reached 0.5 log of CFU/g after 9.05 hours distribution when the initial count was 0.1 log CFU/g (Figure 4.22).

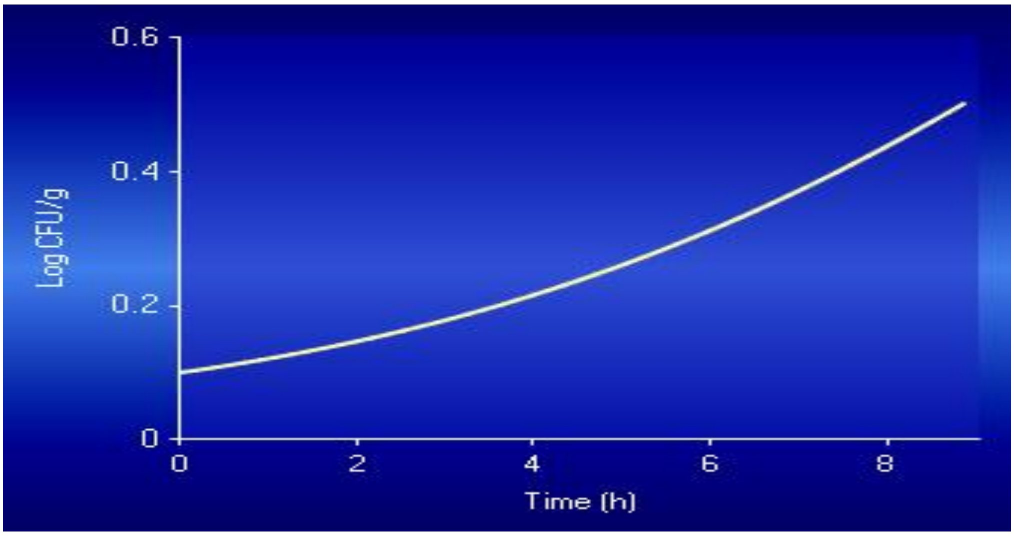


Figure 4.22 Growth prediction of *E.coli* during distribution at 19.5°C, pH:6.42, a_w:0.98 and for 9.05 hours observation period

4.3.2.3 *Pseudomonass* spp. prediction

The result showed that *Pseudomonass* spp. grew rapidly after 4 hours during distribution at 19.5°C (Figure 4.23). The growth rate of *Pseudomonass* spp. was 0.174 log concentration per hour. The doubling time of *Pseudomonass* spp. was 1.73 hours, which means *Pseudomonass* spp. was able to double its population in every 1 hour and 44 minutes during distribution (Table 4.21). *Pseudomonass* spp. reached 1 log CFU/g after 9.05 hours distribution when the initial count was 0.1 log CFU/g (Figure 4.23).

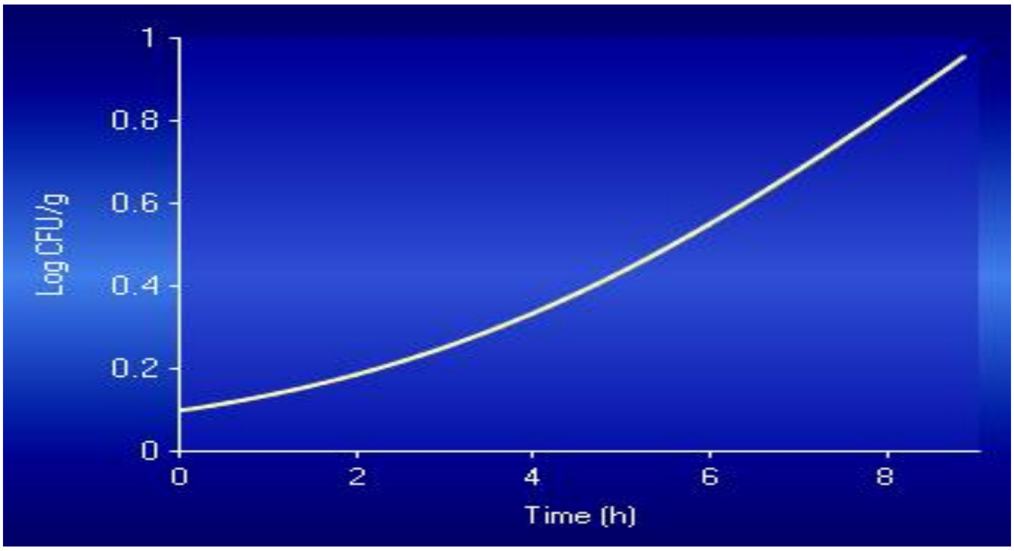


Figure 4.23 Growth prediction of *Pseudomonass* spp during distribution at 19.5°C, pH:6.42, a_w:0.98 and for 9.05 hours observation period

4.3.2.4 *Listeria monocytogenes* prediction

The result showed that *Listeria monocytogenes* grew rapidly after 4 hours during distribution at 19.5°C (Figure 4.24). The growth rate of *Listeria monocytogenes* was 0.169 log concentration per hour. The doubling time of *Listeria monocytogenes* was 1.78 hours which means *Listeria monocytogenes* was able to double its population in every 1 hour and 47 minutes during distribution. *Listeria monocytogenes* reached 0.3 log CFU/g after 9.05 hours distribution when the initial count was 0.1 log CFU/g (Figure 4.24).

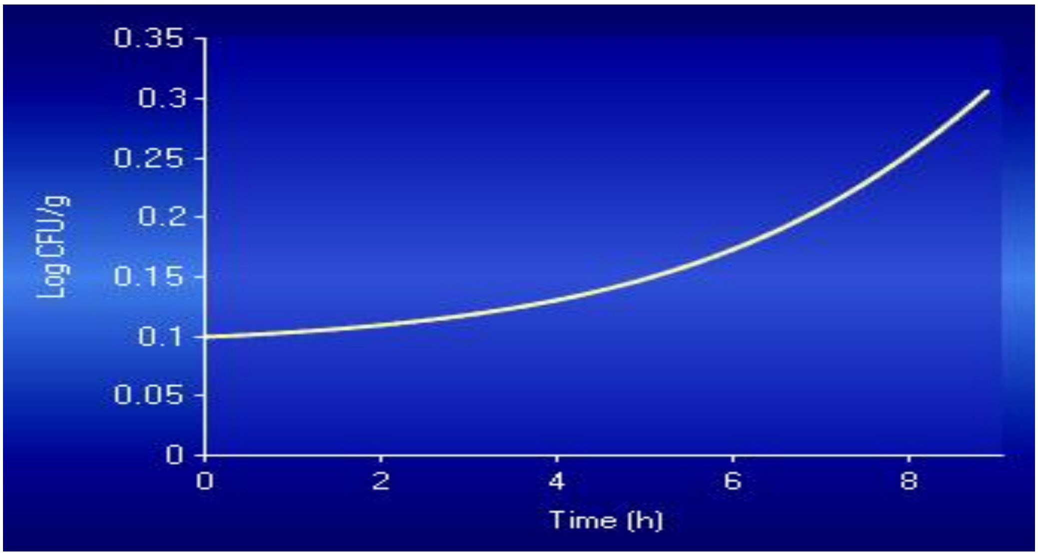


Figure 4.24 Growth prediction of *Listeria monocytogenes* during distribution at 19.5°C, pH: 6.42, a_w :0.98 and for 9.05 hours observation period

4.3.2.5 *Staphylococcus aureus* prediction

The result showed that *Staphylococcus aureus* grew rapidly after 4 hours during distribution at 19.5°C (Figure 4.25). The growth rate of *Staphylococcus aureus* was 0.185 log concentration per hour. The doubling time of *Staphylococcus aureus* was 1.63 hours, which means *Staphylococcus aureus* was able to double its population in every 1 hour and 38 minutes during distribution (Table 4.21). *Staphylococcus aureus* reached 0.6 log CFU/g after 9.05 hours distribution when the initial count was 0.1 log CFU/g (Figure 4.25).

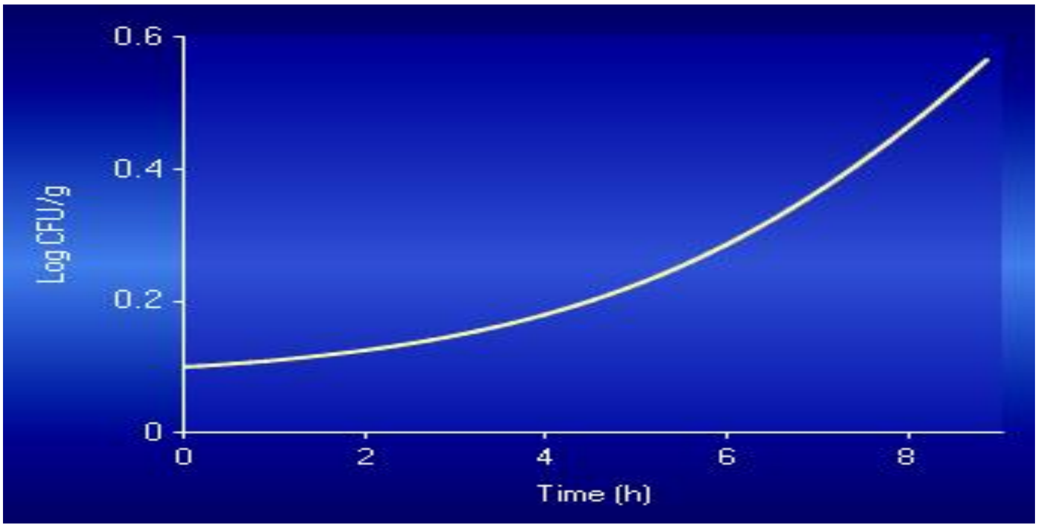


Figure 4.25 Growth prediction of *Staphylococcus aureus* during distribution at 19.5°C, pH: 6.42, a_w :0.98 and for 9.05 hours observation period

4.3.2.6 *Clostridium perfringens* prediction

The result showed that *Clostridium perfringens* grew stagnantly during distribution at 19.5°C (Figure 4.26). The growth rate of *Clostridium perfringens* was 0.081 log concentration per hour. The doubling of *Clostridium perfringens* was 3.74 hours which means *Clostridium perfringens* was able to double the numbers in every 3 hour and 44 minutes during distribution. *Clostridium perfringens* reached 0.11 log CFU/g for 9.05 hours during distribution when the initial count was 0.1 log CFU/g (Figure 4.26).

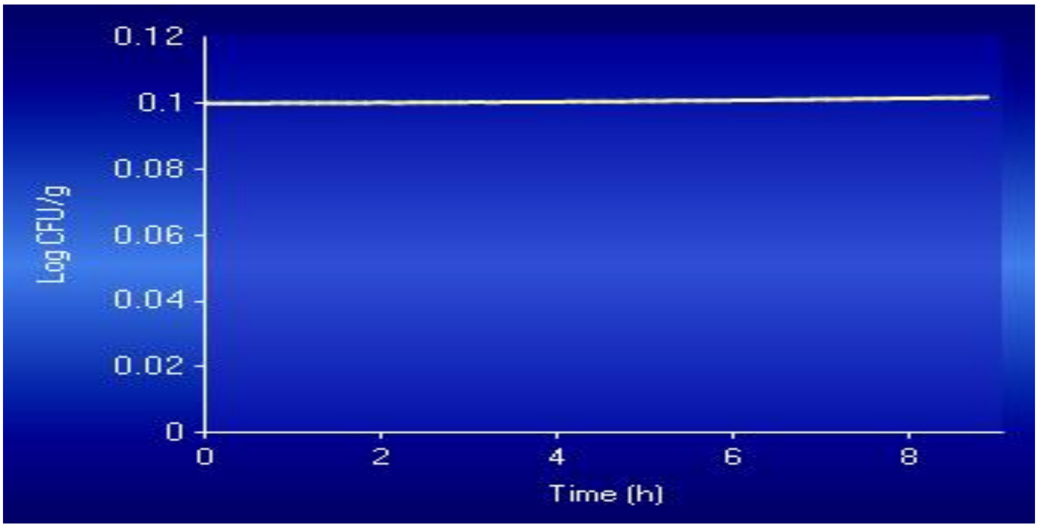


Figure 4.26 Growth prediction of *Clostridium perfringens* during distribution at 19.5°C, pH: 6.42, a_w :0.98 and for 9.05 hours observation period

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 (a_w) 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).

The result from the prediction showed that most of the pathogens grew stagnantly and slowly except for *Pseudomonas* spp. at 8.4 °C in selected parameters during chill storage for 6.3 hours. The number of *Staphylococcus aureus*, *Salmonella* spp. and *Listeria monocytogenes* increased minimally from 0.1 log CFU/g to only 0.11 log CFU/g at 6 hours 18 minutes during chill storage. Meanwhile, the number of *Pseudomonas* spp increased from 0.1 to 0.2 log CFU/g. 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 while *Clostridium perfringens* showed the lowest growth during distribution. 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 a_w 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).

As a conclusion, higher the temperature and longer the observation period could increase the growth rate of selected pathogens on both chill storage and distribution.

CHAPTER V

CONCLUSION

In this survey, the cold chain of chicken sausages was found to be interrupted during two operations; (1) during chill storage (2) during product distribution. During chill storage, temperature fluctuation due to frequent door opening and longer loading time was the major cause of temperature increase up to 11.1°C. During product distribution, longer delivery time and using unrefrigerated truck container caused temperature rise up to 19.5°C. The rise in temperature, in turn, resulted in higher TPC which subsequently increased TA, decreased pH and shear force.

From ComBase® prediction, the growth of selected pathogens based on the worst temperature survey during storage and distribution was performed. The prediction growth showed that most of the pathogens grew stagnantly and slowly except for *Pseudomonas* spp. at 8.4 °C in selected parameters during chill storage for 6.3 hours. The number of *Pseudomonas* spp increased from 0.1 to 0.2 log CFU/g. 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.

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APPENDICES

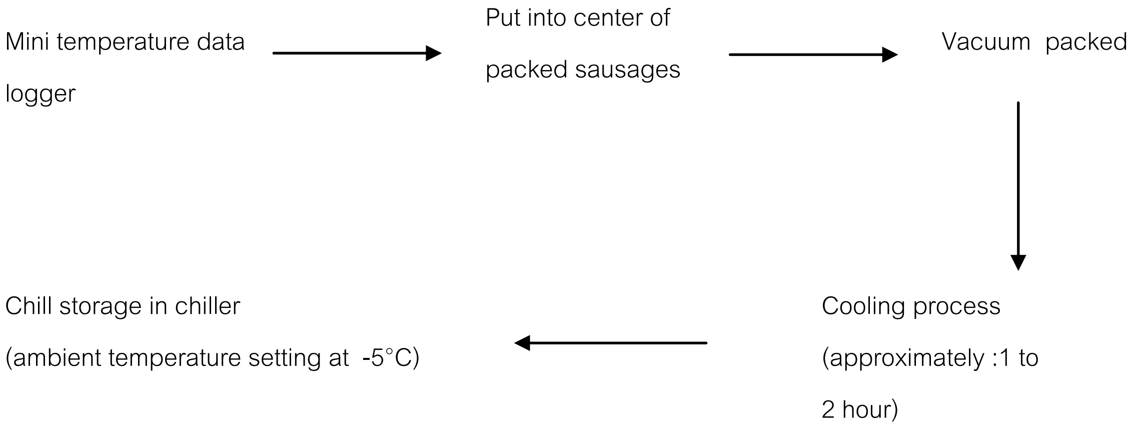
APPENDIX A

A1. Table of microbiology standard of chicken sausages based on Thai Industrial Standard Institute for meat sausages

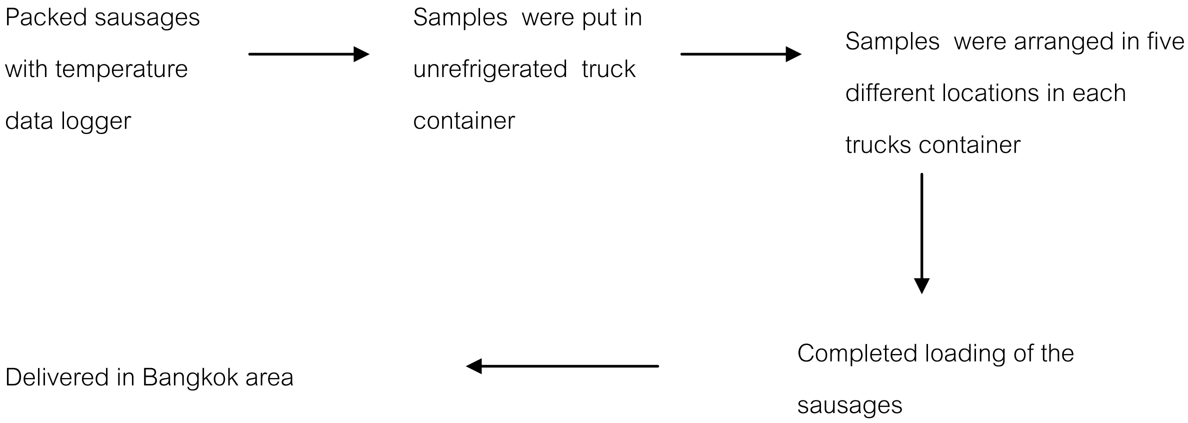
Microbiology Test	Method Reference	Limit per gram
Total plate count (TPC)	AOAC (2000) 17.2.01	Not exceed 10^5
Escherichia coli by MPN test	AOAC (2000) 17.2.02	less than 3g
Salmonella spp.	AOAC (2000) 17.9.01	cannot be found in 25g of sample
Staphylococcus aureus	AOAC (2000) 17.5.02	cannot be found in 0.1g
Clostridium perfringens	AOAC (2000) 17.7.02	cannot be found in 0.01g sample

Source: Thai Industrial Standard Institute for meat sausages (TISI 2300-2549)

A2. Flow chart of the temperature survey during storage:-



A3. Flow chart of the temperature survey during distribution:-



APPENDIX B

ANALYTICAL METHODS

B1. Determination of titratable acidity

Method by (Wang, 2000)

1. Cut sample into small pieces (for solid sample)
2. Accurately weigh 10 gram of sample
3. Add 90 ml of distilled water
4. Blend the sample using commercial blender until homogenize
5. Filter the homogenate of the sample through Whatman filter paper no. 1
6. Take 25 ml of filtrate and titrate with 0.1N sodium hydroxide (1N, NaOH)
7. Titrate until pH of the filtrate reach 8.1 to 8.2
8. The percentage of acid is expressed as ml of 0.1 N NaOH/g sample required to neutralize the filtrate.

Calculating of titratable acidity:-

$$\text{TA (\% acid)} = \frac{\text{V x M or Nx Eqwt x 1000}}{\text{W x 1000}} \text{ x 100}$$

TA= Titratable acidity

V= volume of titrant (ml)

M/N=Molarity or Normality of titrant, usually NaOH (Sodium hydroxide) (mEqml)

Eq.wt.= equivalent weight of predominant acid (mg/mEq) used lactic acid

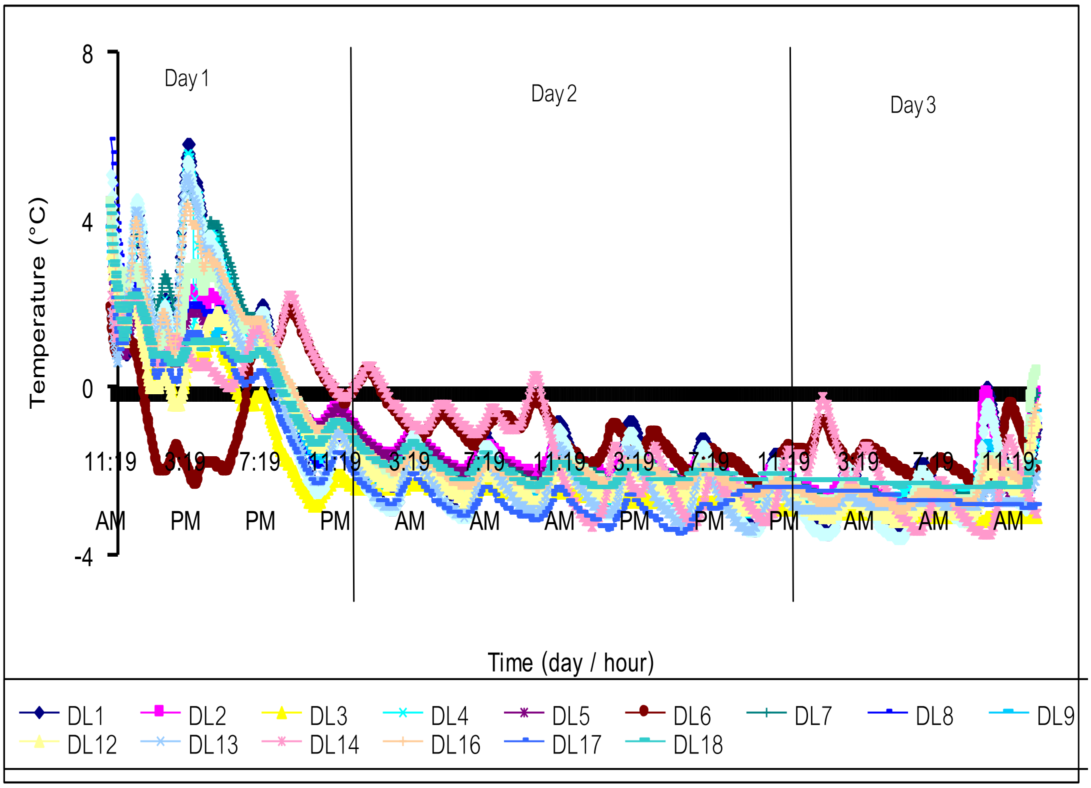
W= mass of sample (g)

1000=factor relating mg to grams (mg/g) (1/10=100/1000)

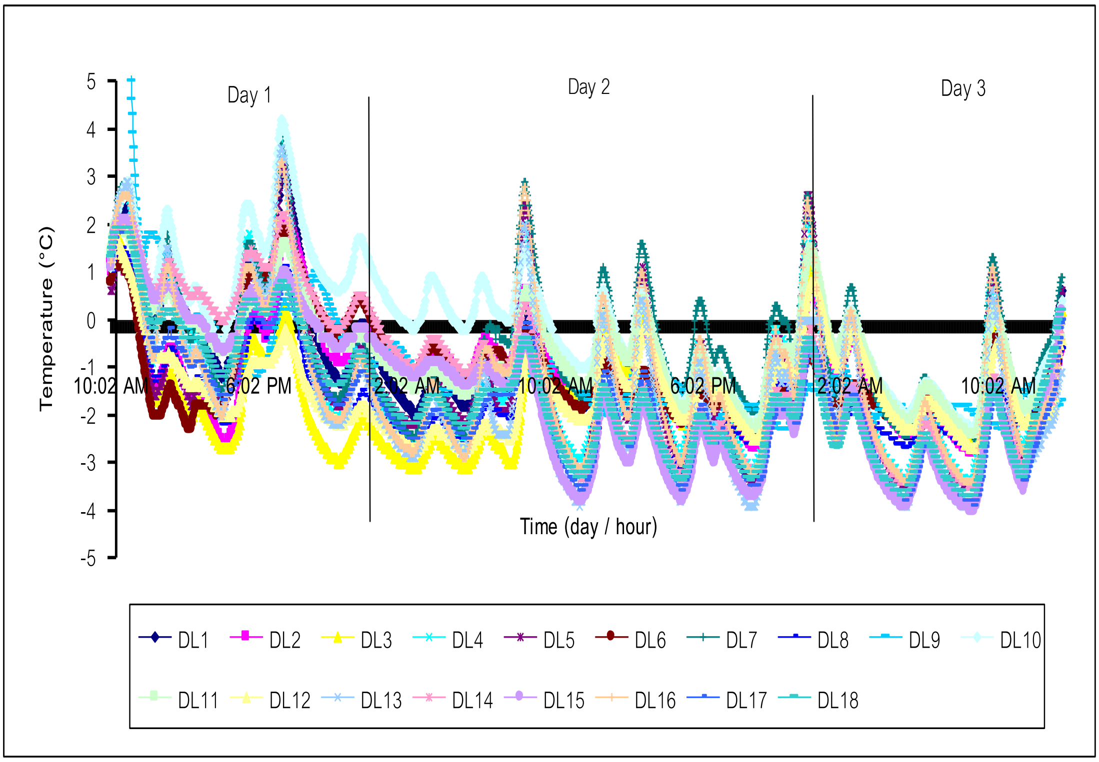
Source: Nielsen 2010

APPENDIX C

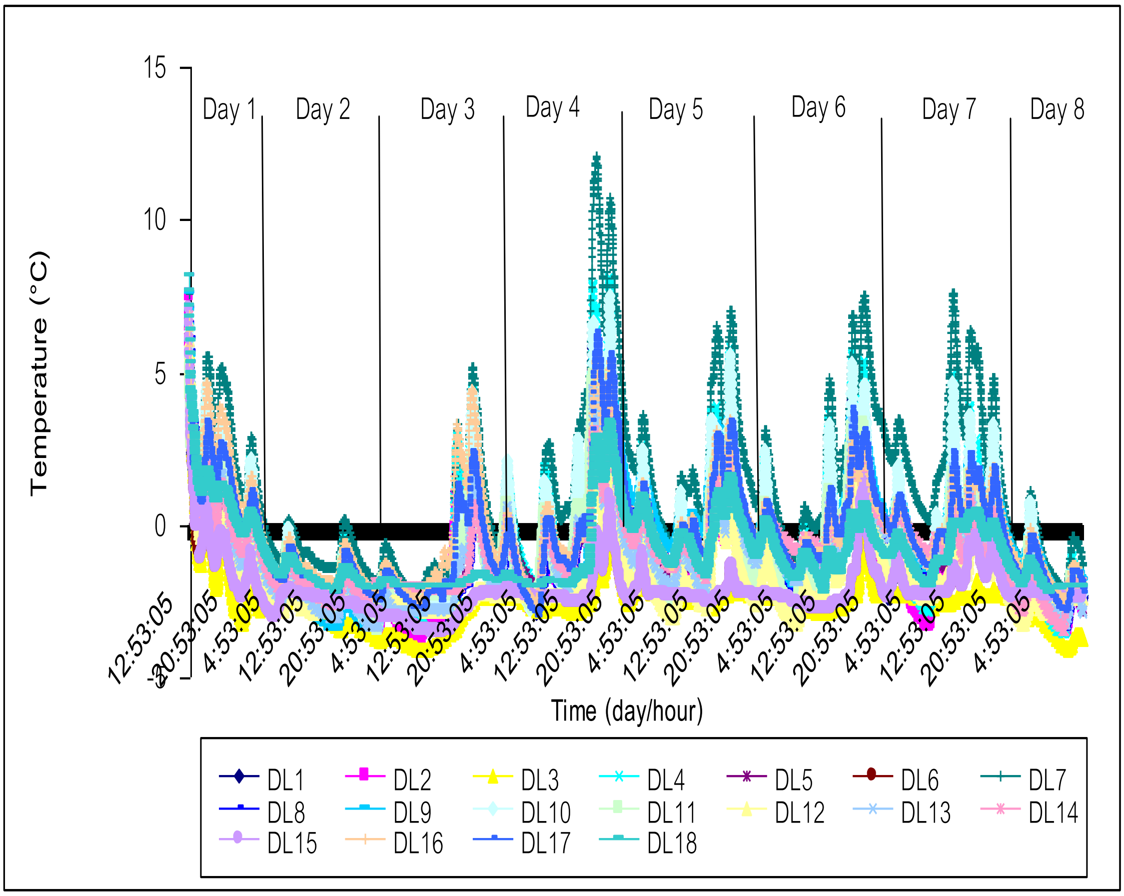
ADDITIONAL DATA



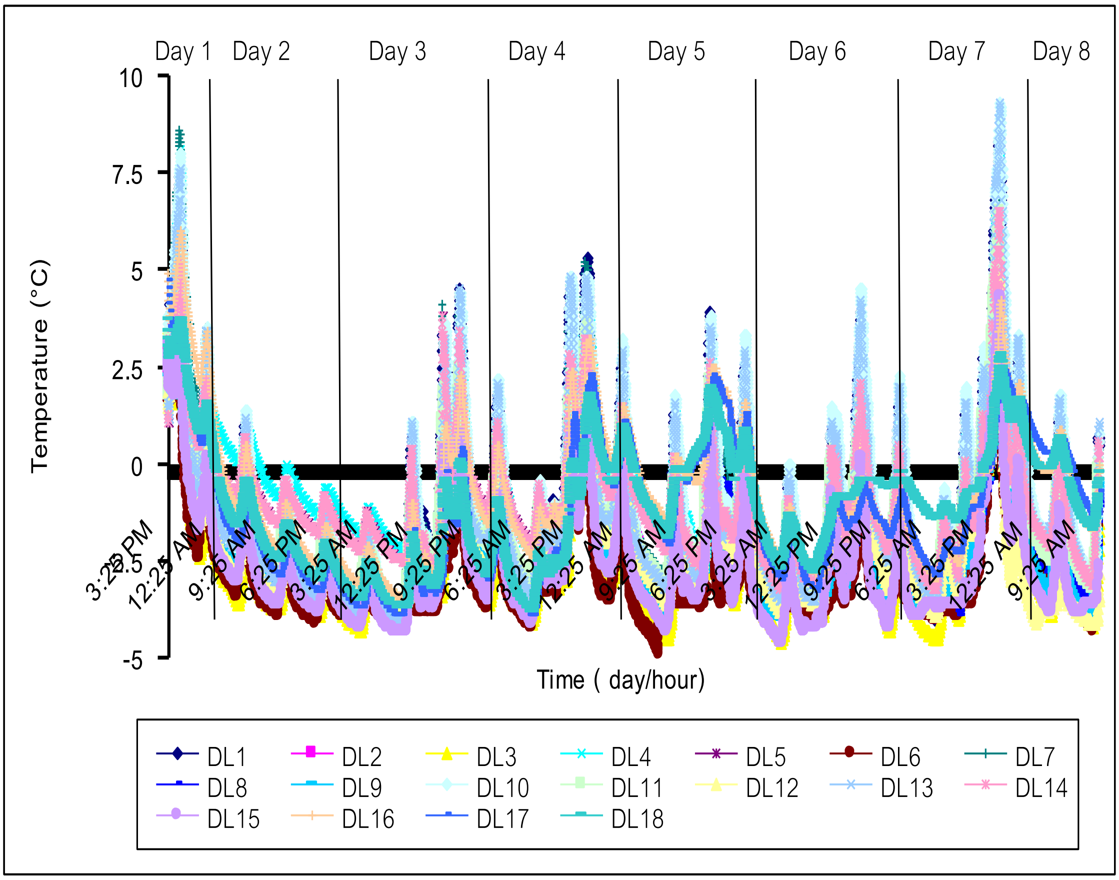
C1. Temperature profile of chicken sausages during chill storage in survey no. 1 (2 days)



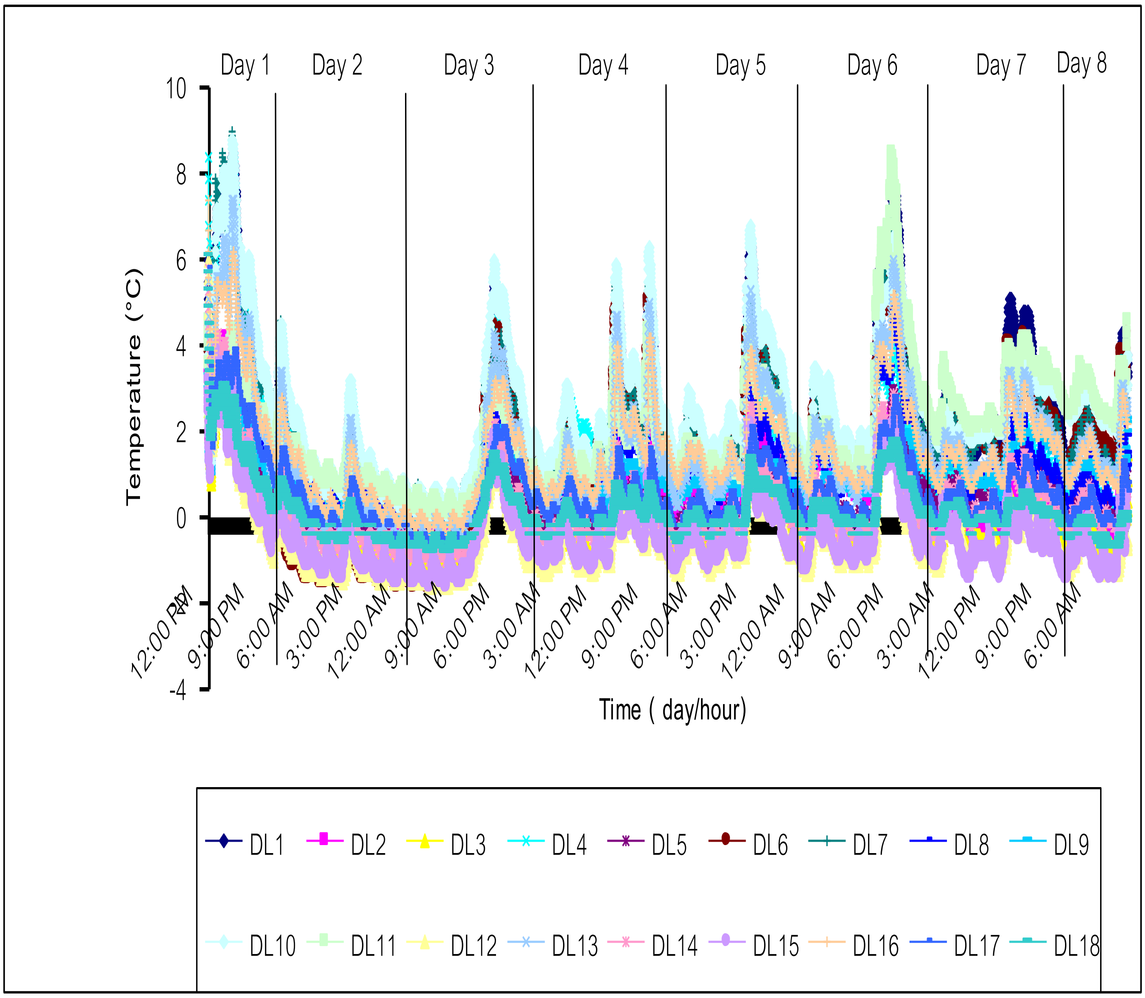
C2. Temperature profile of chicken sausages during chill storage in survey no. 2 (2 days)



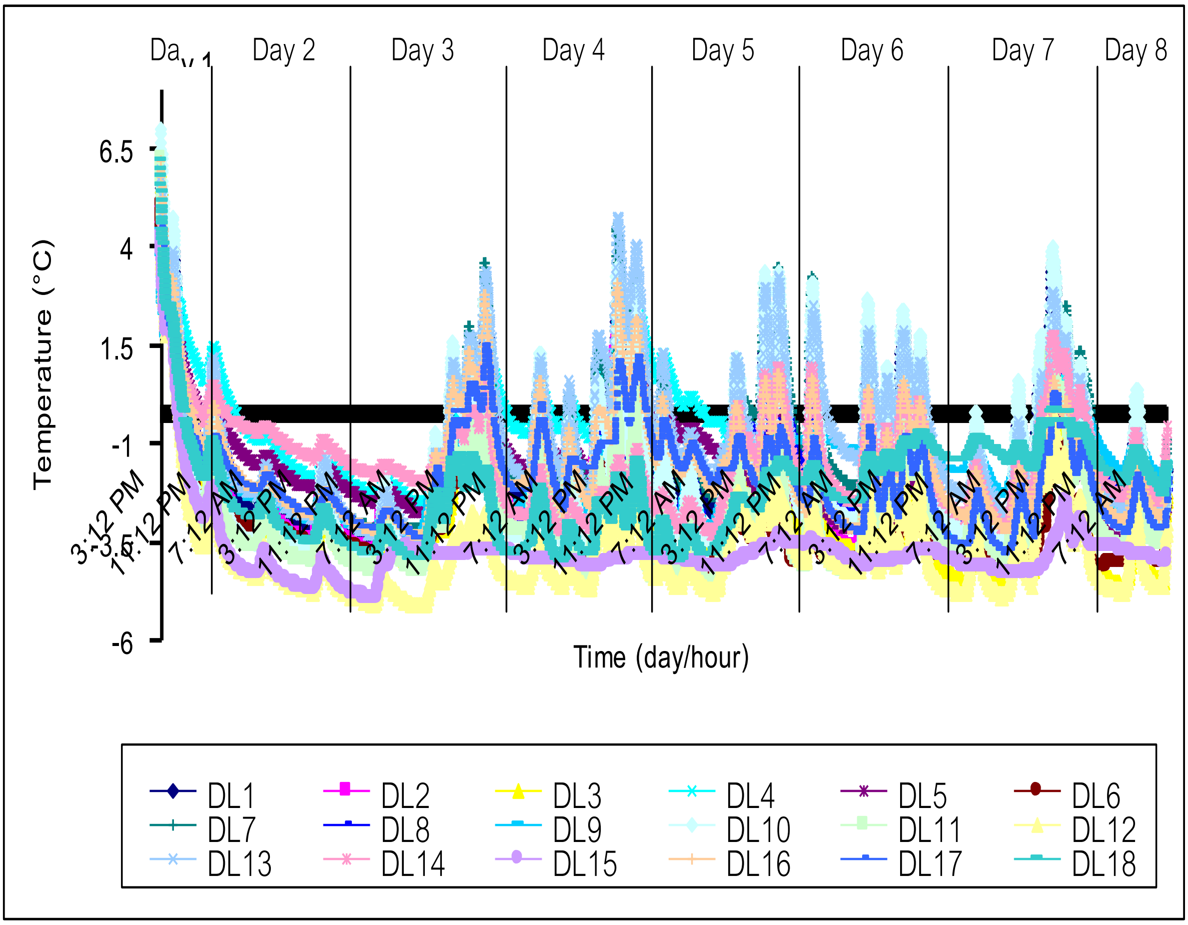
C3. Temperature profile of chicken sausages during chill storage in survey no. 3 (7 days)



C4. Temperature profile of chicken sausages during chill storage in survey no. 4 (7 days)



C5. Temperature profile of chicken sausages during chill storage in survey no. 5 (7 days)



C6. Temperature profile of chicken sausages during chill storage in survey no. 6 (7 days)

VITAE

Ashadi Yaacob was born in Kedah , North State in Malaysia on 26 May 1980. He was graduated from University Putra Malaysia formerly known as Agricultural University of Malaysia on 2003 in Bachelor Degree's of Food Technology. He is working with Food Technology Research Centre in Malaysia Agricultural Research Development Institute (MARDI).

Currently further his study at Chulalongkorn University since 2010 until now in M.Sc. in Food Science and Technology. He also presented a part of his research at 38th Congress on Science and Technology of Thailand (STT 38) organized under the Patronage of His Majesty the King in association with the Faculty of Science, Chiang Mai University, Thailand on 17 to 19 October 2012.

Publication/ Presentation:

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