2. LITERATURE REVIEW

2.1 Background of pork dumplings

Pork dumplings consist of a square sheet of wheat dough enclosing mainly minced pork mixed with ingredients such as sugar, pepper, minced garlic, mushrooms and carrots. To cook these parcels they should be placed in a steamer. Chilled or frozen portions need to be reheated before consumption.

Figure 1 Pork dumplings in different steps of processing

2.2 Production line of pork dumplings

In an industrial production line all the steps are done by machines only. The frozen finished pork dumplings are packed and therefore protected from contamination. Alternatively, local enterprises prepare their home-made dumplings in the kitchen. Because of intense manual activities there is a risk of contamination along the line often connected with poor hygienic conditions.

2.2.1 Industrial processing line

A flow diagram of frozen pork dumplings production is given in figure 2. There are 5 CCPs in the line. CCP 1 is the point of receiving the raw material, CCP 2 represents the process of sieving out foreign body, and CCP 3 represents the heat treatment with a steam conveyer where the pork dumplings are heated until the core temperature reaches 82 ºC. CCP 4 is the freezing step after thermal processing. The pork dumplings are rapidly frozen by air blast at -40ºC, and the core temperature of -18 ºC is reached within 1.5 hours. CCP 5 symbolizes the metal detection stage. The finished product is stored at -15 ºC.

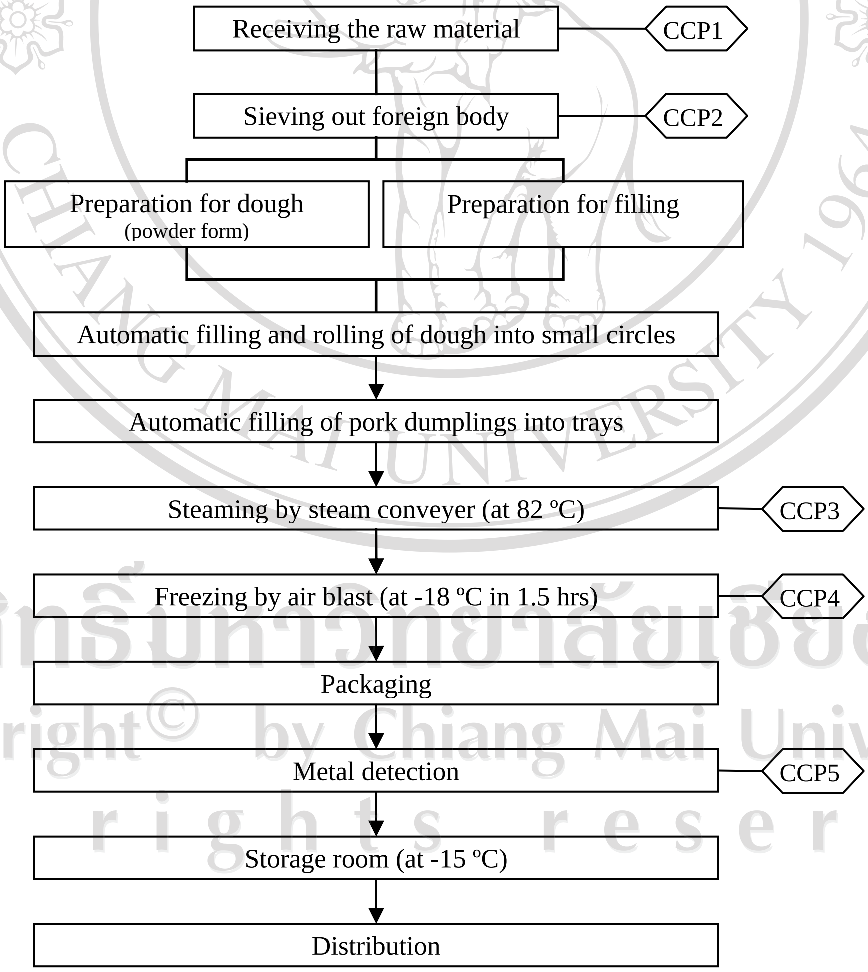

Figure 2 Flow diagram of frozen pork dumplings processing steps

2.2.2 Local enterprise processing line

The hand preparation for pastry is done as follows: put salt, sugar and flour into a bowl and mix it, beat the eggs together and pour them into the flour, knead it until it forms dough, set the dough aside for 20 minutes and cover it with a damp towel to prevent skin forming. The hand preparation of the filling is done encompassing the following steps: soak mushrooms for 5-10 minutes, chop mushrooms, carrots, spring onions, coriander leaves and garlic into small pieces. It is easier to blend the mixture in a food processor. After that, mix the blended ingredient into the minced pork and add the eggs, salt, oyster sauce and cassava starch, and mix well.

For producing the entire pork dumplings: cut the dough into very small balls, roll the dough on a slightly oiled or floured surface into small circles (approximately 6 centimeters diameter), lift up the edges inwards of the dough to form the sides and squeeze the filling into the centre. For getting the best results pleat the edges of the pastry to form pleated sides of the parcels. The pork dumplings are cooked in a steamer for 10 minutes before they are chilled in the refrigerators.

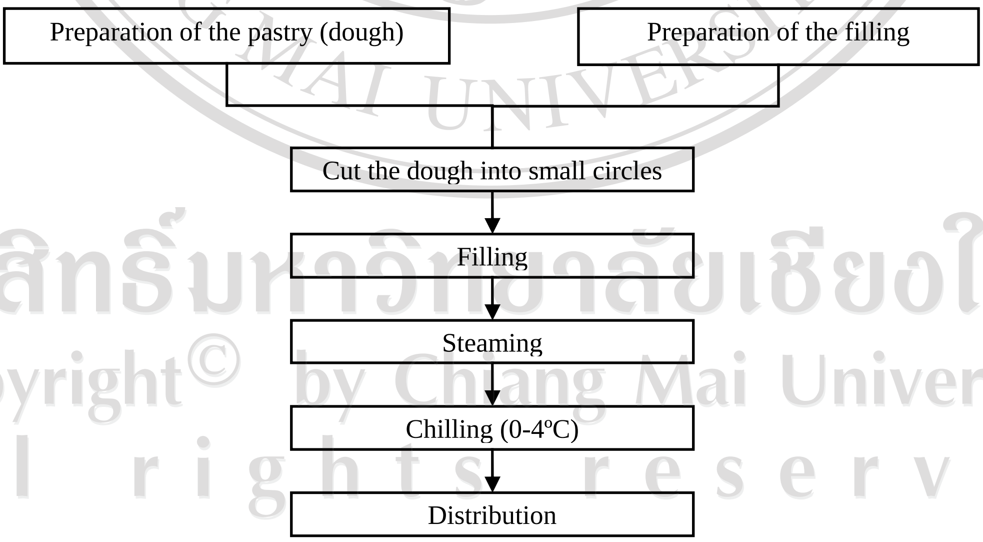

Figure 3 Flow diagram of ready-to-eat pork dumplings processing steps

2.3 Factors Influencing the Microbial Growth

Many factors must be evaluated for each specific food when making decisions whether it needs preventive measures like time/temperature safety controls. These factors can be divided into intrinsic and extrinsic ones. Intrinsic factors are those that are characteristic parameters of the food itself. Extrinsic factors are those that refer to the environment surrounding the food. The need for time/temperature controls primarily depends on the probability of a contamination with pathogenic microorganisms of concern and the potential for subsequent growth and/or toxin production.

2.3.1 Intrinsic parameters

The parameters of plant and animal tissues that are characteristic of the matrix itself are called intrinsic parameter. These parameters are as follows:

1. pH and acidity/buffer capacity
2. Moisture content
3. Oxidation-reduction potential (Eh)
4. Nutrient content
5. Antimicrobial constituents
6. Biological structures

2.3.1.1 pH and acidity/buffer capacity

The natural acidity of a food or the acids produced by fermentation have been used as a preservative ever since ancient times. In a natural state, most foods such as meat, fish, and vegetables belong to the slightly acidic foods, while most fruits are moderately acidic. Very few foods such as egg white are alkaline. The pH can interact with factors such as a_w, temperature, salt, redox potential and preservatives to inhibit the growth of pathogens and other microorganisms. The pH also influences the lethal capacity of the heat treatment of the food. A reduced amount of heat is needed to inactivate microbes when the pH is reduced.

Table 1 pH ranges of some common foods

Product	pH	Product	pH
Dairy products		**Fish and shellfish**	
Butter	6.1-6.4	Fish (most species)	6.6-6.8
Buttermilk	4.5	Clams	6.5
Milk	6.3-6.5	Crabs	7.0
Cream	6.5	Oysters	4.8-6.3
Cheese (American mild and cheddar)	4.9;5.9	Tuna fish	5.2-6.1
Meat and poultry		Shrimp	6.8-7.0
Beef (ground)	5.1-6.2	Salmon	6.1-6.3
Ham	5.9-6.1	White fish	5.5
Veal	6.0		
Chicken	6.2-6.4		
Liver	6.0-6.4		
Pork	5.6-6.2		

Table 2 Approximate pH values permitting the growth of selected pathogens in food

Microorganism		Minimum	Optimum	Maximum
Clostridium perfringens		5.5 – 5.8	7.2	8.0 – 9.0
Vibrio vulnificus		5.0	7.8	10.2
Bacillus cereus		4.9	6.0 –7.0	8.8
Campylobacter spp.		4.9	6.5 –7.5	9.0
Shigella spp.		4.9		9.3
Vibrio parahaemolyticus		4.8	7.8 – 8.6	11.0
Clostridium botulinum	toxin	4.6		8.5
	growth	4.6		8.5
Staphylococcus aureus	growth	4.0	6.0 –7.0	10.0
	toxin	4.5	7.0 – 8.0	9.6
Enterohemorrhagic *Escherichia coli*		4.4	6.0 –7.0	9.0
Listeria monocytogenes		4.39	7.0	9.4
Salmonella spp.		4.2[1]	7.0 –7.5	9.5
Yersinia enterocolitica		4.2	7.2	9.6

Sources: Table 5.3 in ICMSF 1980, p 101.
[1] pH minimum as low as 3.8 has been reported when acidulants other than acetic acid or equivalent are used.

2.3.1.2 Moisture content

Microorganisms need water to grow in food products. The reduction of the moisture content in foods is one of the oldest preservation methods. Food microbiology generally describes the water requirements of microorganisms in terms of the water activity (a_w) which is equivalent to the “biological available water”. Many foods such as fresh meat, vegetables, and fruits have a_w values that are close to the optimum growth level of most microorganisms (0.97-0.99). Table 3 shows the a_w levels of some common food categories. The a_w in foods can be influenced by a number of measures, including the addition of solutes such as salt or sugar, physical removal of water through drying or baking, or binding of water to various macromolecular components in the food.

Microorganisms generally have an optimum and minimum levels of a_w for their growth depending on other growth factors in their environment and their taxonomic classification. For example, Gram negative bacteria are generally more sensitive to a low level of a_w than Gram positive bacteria. It should be noted that many bacterial pathogens are controlled at water activities well above 0.92, and only *S. aureus* can grow and produce toxin below a_w 0.90.

Table 3 a_w values of foods

Animal Products	a_w
fresh meat, poultry, fish	0.99 – 1.00
natural cheeses	0.95 – 1.00
pudding	0.97 - 0.99
eggs	0.97
cured meat	0.87 – 0.95
sweetened condensed milk	0.83
Parmesan cheese	0.68 – 0.76
honey	0.75
dried whole egg	0.40
dried whole milk	0.20

Table 4 Approximate a_w values for growth of selected pathogens in food

Organism		Minimum	Optimum	Maximum
Campylobacter spp.		0.98	0.99	
Clostridium botulinum type E*		0.97		
Shigella spp.		0.97		
Yersinia enterocolitica		0.97		
Vibrio vulnificus		0.96	0.98	0.99
Enterohemorrhagic *Escherichia coli*		0.95	0.99	
Salmonella spp.		0.94	0.99	>0.99
Vibrio parahaemolyticus		0.94	0.98	0.99
Bacillus cereus		0.93		
Clostridium botulinum types A & B**		0.93		
Clostridium perfringens		0.943	0.95-0.96	0.97
Listeria monocytogenes		0.92		
Staphylococcus aureus	growth	0.83	0.98	0.99
	toxin	0.88	0.98	0.99

ICMSF 1996.
* *proteolytic; * non-proteolytic

2.3.1.3 Oxidation-reduction Potential

The oxidation-reduction or redox potential (Eh) refers to the ratio of the total oxidizing (electron accepting) power to the total reducing (electron donating) power of the substance. Redox potential is measured in terms of millivolts and characterizes whether a substance gains or loses electrons. The major groups of microorganisms classified according to their relationship to their Eh for growth are aerobes, anaerobes, facultative aerobes and microaerophiles. Generally, the ranges at which different microorganisms can grow are as follows: aerobes +500 to +300 mV, facultative anaerobes +300 to -100mV, and anaerobes +100 to less than -250 mV. For example, *C. botulinum* is a strict anaerobe that requires an Eh of less than +60 mV for growth; however, a slower growth can occur at higher Eh values.

The redox potential behaves highly variable depending on changes in the pH of the food, microbial growth, packaging, the partial pressure of oxygen in the storage environment and ingredients and the composition (protein, ascorbic acid, reducing sugars, oxidation level of cations and so on).

Table 5 Redox potentials of some foods

FOOD	Presence of air	Eh (mV)	pH
Milk	+	+300 to +340	NR
Cheese			
Cheddar	+	+300 to -100	NR
Dutch	+	-20 to -310	4.9–5.2
Emmenthal	+	-50 to –200	NR
Butter serum	-	+290 to +350	6.5
Egg (infertile after 14 d)	+	+500	NR
Meats			
Liver, raw minced	-	-200	~7
Muscle			
Raw, post-rigor	-	-60 to -150	5.7
Raw, minced	+	+225	5.9
Minced, cooked	+	+300	7.5
Cooked sausages and canned meat	-	-20 to –150	~6.5
Cereals			
Wheat (whole grain)	-	-320 to -360	6.0
Wheat (germ)			
Barley (ground)	-	-470	NR
	+	+225	7
Potato tuber	-	~ -150	~6
Plant juices			
Grape	-	+409	3.9
Lemon	-	+383	2.2
Pear	-	+436	4.2
Spinach	-	+74	6.2
Canned foods			
"Neutral"	-	-130 to -550	>4.4
"Acid"	-	-410 to -550	<4.4

NR = Not reported
Reproduced from Mossel and others 1995, p 185 by permission of D.A.A. Mossel.

2.3.1.4 Nutrient Content

Microorganisms require basic nutrients for their growth and the maintenance of metabolic functions. These nutrients include water, nitrogen, a source of energy, vitamins and minerals. The content of these nutrients varies from food to food. Meats have abundant protein, lipids, minerals and vitamins but low levels of carbohydrates. Foods such as milk and milk products and eggs are rich in nutrients.

Many microorganisms derive their energy from carbohydrates, alcohols and amino acids. They can metabolize simple sugars such as glucose. Other bacteria can metabolize more complex carbohydrates such as starch or glycogen found in meat and meat products. Some microorganisms are able to metabolize peptides and more complex protein to gain amino acids which serve as a source of nitrogen and energy. Other sources of nitrogen include, for example, urea, ammonia and creatinine. Fats are rarely used as a source of energy.

2.3.1.5 Antimicrobial constituents

Some foods contain natural antimicrobial compounds, for example eugenol in cloves, allicin in garlic, lactoferrin, conglutinin and the lactoperoxidase system in cow's milk, lysozyme in eggs and milk, and other compounds in fresh meat, poultry and seafood. Gram negative psychotrophs such as the *Pseudomonadaceae* have been shown to be very sensitive to the lactoperoxidase system.

2.3.1.6 Biological structures

Plant and animal derived foods, especially in the raw state, have biological structures that may prevent the entry and growth of microorganisms. Examples of such physical barriers include shell of seeds and nuts, skin of fruits and vegetables, animal hide, cuticle, shell, and membranes of eggs. When stored under proper conditions of humidity and temperature the outer shell and membrane of eggs prevent the entry of nearly all microorganisms. The skin covering fish and meats such as beef and pork prevents the contamination and spoilage of these foods, partly because it tends to dry out faster than freshly cut surfaces.

2.3.2 Extrinsic parameters

The extrinsic parameters of foods are not substrate dependent. They represent those properties of the storage environment that affect both the foods and their microorganisms. Those of greatest importance are as follows;

1. Effect of time/temperature
2. Relative humidity of environment
3. Presence and concentration of gases

2.3.2.1 Effect of time/temperature

Microorganisms have a temperature range in which they grow. At low temperatures, reaction rates for the individual enzymes in the organism become slower. Additionally the fluidity of the cytoplasmic membrane, interfering with transport mechanisms, is reduced. At high temperatures, the structure of the cell becomes denatured, and then inactivation of heat-sensitive enzymes occurs.

Four major groups of microorganisms have been described based on their temperature ranges for growth: thermophiles, mesophiles, psychrophiles, and psychrotrophs. The optimum temperature for the growth of thermophiles lies between 55 to 65 °C (131 to 149 °F) with the maximum as high as 90 °C (194 °F) and a minimum of around 40 °C (104 °F). Mesophiles, which include virtually all human pathogens, have an optimum growth range between 30 °C (86 °F) and 45 °C (113 °F), and a minimum growth temperature ranging from 5 to 10 °C (41 to 50 °F). Psychrophilic organisms have an optimum growth range from 12 °C (54 °F) to 15 °C (59 °F) with a maximum range of 15 °C (59 °F) to 20 °C (68 °F). There are very few true psychrophilic organisms of importance to foods. Psychrotrophs such as *L. monocytogenes* and *C.botulinum* type E are capable of growing at low temperatures (minimum of - 0.4 °C [31 °F] and 3.3 °C [38 °F], respectively), but have a higher growth optimum (37 °C [99 °F] and 30 °C [86 °F], respectively) than true psychrophiles. Psychrotrophic organisms are much more relevant to foods and include spoiling bacteria, yeasts and molds, as well as certain food-borne pathogens.

In addition to the effect of temperature as such, the time of exposition to a certain temperature needs critical consideration. Food producers or manufacturers realized the concept of time as it relates to microbial growth when a product's shelf life is determined. Shelf life is the time period from the moment when the product is produced until the time it is used by consumers. Several factors are used to characterize a product's shelf life, ranging from organoleptic qualities to microbiological safety.

Table 6 Temperature ranges for prokaryotic microorganisms

Group	Temperature °C (°F) Minimum	Optimum	Maximum
Thermophiles	40 - 45 (104 – 113)	55 - 75 (131 – 167)	60 – 90 (140 – 194)
Mesophiles	5 - 15 (41 – 59)	30 - 45 (86 – 113)	35 – 47 (95 – 117)
Psychrophiles	-5 - +5 (23 – 41)	12 - 15 (54 – 59)	15 – 20 (59 – 68)
Psychrotrophs	-5 - +5 (23 – 41)	25 - 30 (77 – 86)	30 – 35 (86 – 95)

Source: Table 1.1 in ICMSF 1980, p 4.

2.3.2.2 Relative humidity of environment (RH)

The relative humidity of the environment surrounding the food is an important factor because it affects the water activity of the food. If the relative humidity is low, moisture may be drawn from the food into the surrounding air to produce equilibrium. In this case the water activity is reduced, descending from the surface to the core.

Nevertheless, foods should be stored under conditions of low relative humidity if they are likely to obtain surface spoilage from molds, yeasts and certain bacteria. Improperly wrapped meats such as whole chickens and beef cuts tent to suffer intensive surface spoilage in the refrigerator before a greater spoilage occurs, which is due to the generally high RH of the refrigerator combined with the fact that microbial meat-spoilage is essentially aerobic in nature.

Although it is possible to lessen the chances of surface spoilage in certain foods by storing them under low conditions of RH, it should be remembered that the food itself will lose moisture to the atmosphere under such conditions and will thus become undesirable. In selecting the proper environmental conditions of RH both effects should be considered: the possibility of surface growth on the one hand, and the desirable quality to be maintained in the foods in question on the other hand. By altering the gas atmosphere (compare 2.3.2.3), it is possible to retard surface spoilage without lowering the RH.

2.3.2.3 Presence and concentration of gases in the environment

Gases inhibit microorganisms by two mechanisms. First, they can have a direct toxic effect that can inhibit growth and proliferation. Carbon dioxide (CO_2), ozone (O_3) and oxygen (O_2) are gases that act directly as toxins to certain microorganisms. Oxidizing radicals generated by O_3 and O_2 are highly toxic to anaerobic bacteria and can have an inhibitory effect on aerobes depending on their concentration. Carbon dioxide is effective against obligate aerobes, and at high levels it can restrain other microorganisms. A second inhibitory mechanism is achieved by modifying the gas composition, which has indirect inhibitory effects by changing the ecology of the microbial environment. When the atmosphere is altered, the competitive environment also altered. Atmospheres that have a negative effect on the growth of one particular microorganism may promote the growth of another one. This effect may have positive or negative consequences depending upon the native pathogenic microflora and the substrate. The replacement of oxygen by nitrogen is an example of this indirect antimicrobial activity.

Modern technologies in this field include modified atmosphere packing (MAP), controlled atmosphere packaging (CAP), controlled atmosphere storage (CAS), direct addition of carbon dioxide (DAC) and hypobaric storage.

2.4 Determination of microbial numbers, growth and activity in meat

2.4.1 Aerobic Plate Count

Belonging to the culturing techniques or colony counting techniques Aerobic Plate Count (APC)) is used to determine the number of microorganisms in a food product or an ingredient. These data are often used as indicators of food hygienic quality or predictors for the shelf life of a product. The media used for APC-determination do not contain any selective or differential additives. The colonies counted after inoculation and incubation of the sample aliquots only represent the organisms that could multiply at the given conditions of growth (temperature, incubation period, media, and atmosphere). Other populations of organisms that can only grow at higher or lower temperatures or that grow very slowly or require additional nutritional components or need a specialized atmosphere (such as a reduction in O_2 or increase in CO_2 or N_2) will not be part of the APC.

2.4.2 Lactic acid bacteria

The lactic acid bacteria, such as *Lactobacillus spp.*, *Leuconostoc*, and *Pediococcus*, are facultative anaerobic. The type of spoilage produced by the lactic acid is determined by the nature of the bacteria. Homofermentative lactic acid bacteria produce primarily lactic acid during sugar fermentation. Heterofermentative lactic acid bacteria produce acetic and formic acid, ethanol, and carbon dioxide, in addition to lactic acid. Lactic acid bacteria can spoil a variety of foods, including milk and milk products, meats, vegetables, fruit juices, sugary products, alcoholic beverages and products preserved with vinegar. They are also capable in producing off-flavours when causing spoilage.

2.4.3 Indicators Microorganism of Food Hygiene

Staphylococcus spp.
Pseudomonas spp.
Enterobacteriaceae
Coliforms

2.4.3.1 *Staphylococcus spp.*

The genus Staphylococcus consists of cluster-forming Gram-positive cocci. The main pathogen within the genus, *Staphylococcus aureus*, causes a wide range of major and minor infections in man and animals. Currently there are about 27 different species of staphylococci. These fall into two main groups on the basis of their ability to clot blood plasma by action of the enzyme coagulase. *Staphylococcus aureus* is the most important coagulase-positive species. The coagulase-negative staphylococci are skin commensally, which are now recognized as important opportunistic pathogens that can cause infections associated with artificial limbs, catheters, implants (*S. epidermidis*), and urinary tract infections (*S. saprophyticus*).

Staphylococci, being resistant to dry conditions and high salt concentrations, are well suited to their ecological niche, which is the skin surface. Approximately 30% of all healthy people are “carriers” of *S. aureus*; and even higher number carry coagulase-negative staphylococci.

Staphylococci are commonly present on man and animals, including farm animals used for milk production, (cattle, sheep and goats; mastitis caused by *S. aureus* is a common and costly complication of milking). The contamination of foods can occur from either human or animal sources. Such kinds of contamination may result in staphylococcal food poisoning.

Staphylococcal food poisoning is the name of the condition cause by the enterotoxins which some strains of *S. aureus* produce. The onset of symptoms is usually rapid and in many cases acute, depending on the individual susceptibility to the toxin, the amount of toxin in the food ingested, and the general health of the victim. The most common symptoms are nausea, vomiting, retching, abdominal cramping, and prostration. Some individuals may not always demonstrate all the symptoms associated with the illness. In more severe cases, headache, muscle cramping, and transient changes in the blood pressure and pulse rate may occur. The recovery generally takes two or three days sometimes longer in severe cases.

Five types of enterotoxin (types A-E) are commonly produced by up to 65% of strains of *S. aureus*, sometimes singly and sometimes in combination. These toxic proteins are heat-stable, withstanding exposure to 100 ºC for several minutes. Infective dose: a toxin dose of less than 1.0 microgram in contaminated food will produce symptoms of staphylococcal intoxication. This toxin level is reached when *S. aureus* populations exceed 100,000 per gram.

Foods that are frequently incriminated in staphylococcal food poisoning include meat and meat products; poultry and egg products; salads such as egg, tuna, chicken, potato, and macaroni, bakery products such as cream-filled pastries, cream pies, and chocolate éclairs; sandwich fillings, and milk and dairy products. Foods that are kept at slightly elevated temperatures after heating and require considerable handling during preparation (post processing contamination) are frequently involved in staphylococcal food poisoning. Another kind of food often involved in this intoxication is matrices with a low activity of water.

2.4.3.2 *Pseudomonas spp.*

Pseudomonas spp. which are aerobic, are among the most common spoilage agents of refrigerated foods (Marth, 1998). *Pseudomonas spp.* is Gram-negative psychrotrophs, which grow, although slowly, at refrigeration temperatures (below 7 ºC) but grow optimally at temperatures above refrigeration, e.g. 25-30 ºC. Their maximum growth temperatures are 30-35 ºC. Growth of *Pseudomonas spp.*, like that of other Gram-negative psychrotrophs, is effected by oxygen tension, salt and other food additives, water activity (a_w), pH and other factors. During growth, Pseudomonads produce protease and lipases that can catalyze reactions causing degradation of protein and fat. The consequence of these reactions is formation of peptides and fatty acids of undesirable flavor (e.g., bitterness, rancidity) and odor. Sometimes these bacteria also produce unsightly green pigments.

The genus Pseudomonas comprises more than 200 species, mostly saprophytes found widely in soil, water and other moist environments. A few species are pathogenic for plants, insects and animals. *Pseudomonas aeruginosa* is the species most commonly associated with human diseases but *Ps. mallei* and *Ps. pseudomallei* are also important pathogens in some parts of the world. Several other species of *Pseudomonas* and a number of other glucose non-fermenters are occasionally isolated from human clinical specimens as opportunistic pathogens. The reasons for the preeminent status of *Ps.auruginosa* as an opportunistic pathogen lies in its adaptability, its innate resistance to many antibiotics and disinfectants, its varied armory of putative virulence factors, and in an increasing number of patients compromised by age, underlying disease or immunosuppressive therapy.

2.4.3.3 *Enterobacteriaceae*

The *Enterobacteriaceae* are a large, heterogeneous group of Gram-negative rods whose natural habitat is the intestinal tract of humans and animals. The family includes many genera (eg, *Escherichia, Shigella, Salmonella, Enterobacter, Klebsiella, Serratia, and Proteus*). Some enteric organisms, i.e. *Escherichia coli*, are part of the normal flora and incidentally cause diseases, while others, the *Salmonellae* and *Shigellae*, are regularly pathogenic for humans.

The *Enterobacteriaceae* are facultative anaerobes or aerobes which ferment a wide range of carbohydrates, possess a complex antigenic structure, and produce a variety of toxins and other virulence factors. The family *Enterobacteriaceae* is characterized biochemically by the ability to reduce nitrates to nitrites and to ferment glucose with the production of acid or acid and gas. The *Enterobacteriaceae* do not require increased amounts of sodium chloride for growth and are oxidase-negative.

The *Enterobacteriaceae* are useful indicators of hygiene and post-processing contamination of processed foods. Their presence in high number in foods indicates that an unacceptable level of contamination has occurred or there has been underprocessing (e.g. inadequate cooking). Testing for the *Enterobacteriaceae* is not applicable to fresh fruits and vegetables or foods containing these because fresh fruit and vegetables often carry high levels of these organisms as part of their normal flora.

2.4.3.4 Coliforms

Coliforms are a general term for facultative Gram-negative rods that inhabit the intestinal tracts of humans and animals without usually causing diseases. *Escherichia coli* are normally an organism of the bowels, as are probably some of the *Klebsiella*, whereas the saprobes *Enterobacter*, *Serratia* and *Citrobacter* groups occur infrequently in the intestines.

Fecal coliforms are bacteria that ferment lactose to produce acid and gas at 44.5 °C up to 48 hours. They include the genera *Escherichia*, *Enterobacter* and *Klebsiella*, and they are used as indicator organisms in water quality testing. Although the bacteria are not pathogenic, they are a common indicator of feacal contamination and a possible presence of other pathogens. In general, increased levels provide a warning of failure in water treatment, a break in the integrity of the processing and distribution system, or possible contamination with pathogens. When the levels are high, there may be an elevated risk of gastroenteritis.