Chapter 2

Review of Literature

This chapter reviews the fermentation of meat by the use of starter culture technology. The characteristic and function of lactic acid bacteria in fermented sausage are reported. It includes the biological changes such as cured meat pigment, protein, and flavor. In addition, the study of mathematical modeling in fermented sausage is described.

2.1 Fermentation of meat

Fermentation of meat, a worldwide and oldest technique in food preservation, was originally used to store food for longer times (Hutkins, 2006). This principle is not only employed to ensure microbiological safety, but also to enhance the flavour and nutritional quality of product. The technique allows a low energy conservation of meat and remains to be considered as yielding a high quality product. Traditional fermentation relies on natural contamination by environmental microflora. During fermentation, complex biochemical and physical reactions take place resulting in a significant change of sensorial characteristics. Fermentation causes an increase in organic acids along with a concomitant decrease in pH due to the fermentation of added carbohydrates, i.e., sugar. The primary fermentation product, lactic acid, served to lower the pH and contributed to the stability of these sausages against food-borne pathogens and other undesirable microorganisms. Besides lactic acid, there are a variety of other products that are formed during the fermentation process. These include organic acids, carbon dioxide and alcohols that give distinct flavour and texture of the fermented products. Therefore, fermentation is a biological process and it is influenced by many factors that need to be controlled in order to produce a safe and consistent product quality.

2.1.1 Thai fermented sausage

The main ingredients used in Thai fermented sausages are meat, salt, nitrite or nitrate, sugar or cooked rice, and spices. The mixtures are mixed well and stuffed into casing. Natural fermentation is at ambient temperature (about 30°C) for 3 to 4 days by predominant bacteria, lactic acid bacteria until becoming sour and cooked before eating (Thai Industrial Standards Institute, 1994). The fermentable carbohydrates are used as carbon sources for the sausage microflora to increase acidity of meat by producing organic acids, mainly lactic acid (Leroy and De Vuyst, 2004). The pH drop caused by organic acids production prevents the growth of spoilage and pathogenic bacteria such as *Salmonella*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Escherichia coli* O157:H7. Sodium chloride added in the raw sausage mixtures affects microorganism growth, interacts with the myofibrillar, solubilizes proteins, and contributes obviously to the taste of meat products (Yada, 2004). The general consensus of addition of nitrate or nitrite salt to meats is positive contribution to the color, flavor and shelf life of the product. The curing step has been employed in traditionally made fermented sausages to increase the number of lactic acid, color and flavor forming bacteria (Olesen et al., 2004). Nitrite and nitrate salts also inhibit the growth of unwanted microorganisms, particularly *Clostridium Botulinum* spores which can create a lethal toxin. Nitrite and nitrate salts should be added at a maximum of 125 ppm (parts per million) and 500 ppm, respectively (Retrived January 20, 2010, from http://elib.fda.moph.go.th/). Spices, such as pepper and garlic, have an impact on flavour and they may also have antioxidative and antimicrobial effect (Hammes and Knauf, 1994). On the other hand, sausage production depends on natural fermentation; therefore, product quality varies from batch to batch. Unfortunately, if the normal beneficial microflora does not multiply as usual, the product may spoil. It can cause illness due to pathogenic microorganisms or their toxins, and even become lethal due to botulinum toxin production (Woodburn, 1992). It is considered a dangerous process due to the lack of fermentation control. To prevent these problems, the use of starter cultures has become worldwide application in food fermentation.

2.2 Starter culture

Starter culture is a concentrated and selected bacteria that has been used in the production of sausages. A starter culture can provide particular characteristics in a more controlled and predictable fermentation. The use of starter cultures means that the proper type of bacteria in the required amount is added to the sausage emulsion to ensure efficient and safe fermentation. Meat starter cultures are selected bacteria that have been isolated from the meat, purified, and grown in large numbers under controlled conditions. Then they are concentrated and preserved before used in meat fermentation (Bonomo et al, 2008). Consequently, a microenvironment was provided for those microorganisms that were not only salt tolerant, but also could grow in the absence of air. The identification and characterization allows to search potential protective cultures to be used in the meat product, especially lactic acid bacteria that have a good potential to be used for bio-protection of meat product. Klingberg et al. (2005) identified potential probiotic cultures suitable as starter cultures from meat products. *Lactobacillus plantarum* as well as *L. pentosus* were successfully applied as starter cultures for the manufacture of the Scandinavian-type fermented sausage. In the study of Lee et al., (2006), *L. plantarum* isolated from kimchi had an ability to adapt to the complex environment of fermented sausage, which will allow them to act as starter cultures and natural preservatives in sausage production. The direct addition of selected starter cultures to raw materials improves the quality and safety of the end product and standardizes the fermentation process (Campbell-platt and Cook, 1995; Lücke, 2000). The microorganisms that are primarily involved in sausage fermentation include species of lactic acid bacteria (LAB), gram-positive, catalase-positive cocci (GCC), molds and yests. In addition, starter cultures can be classified into the following groups; (1) lactic acid producing cultures, (2) color fixing and flavor forming cultures, (3) surface coverage cultures; and (4) bio-protective cultures or bacteriocin-producing starter cultures (Leroy et al. , 2006). Bacteriocins are some kind of antibiotics which kill unwanted bacteria. Some of the lactic acid cultures, i.e., *Pediococcus* possess antimicrobial properties which are very effective in growth inhibition of *S. aureus*, *Salmonella*, *Cl. botulinum* and other microorganisms, including yeasts.

The most important microorganisms used in starter cultures are presented in Table 2.1. The advantages of starter cultures are complete fermentation, control of fermentation rate, reduction of fermentation time, reduction of formation of off-flavours, formation of more consistent flavor characteristics and improvement of quality and profitability. Starter cultures also provide safety by competing for food with undesirable bacteria thus inhibiting their growth (Hugas and Monfort, 1997).

Table 2.1

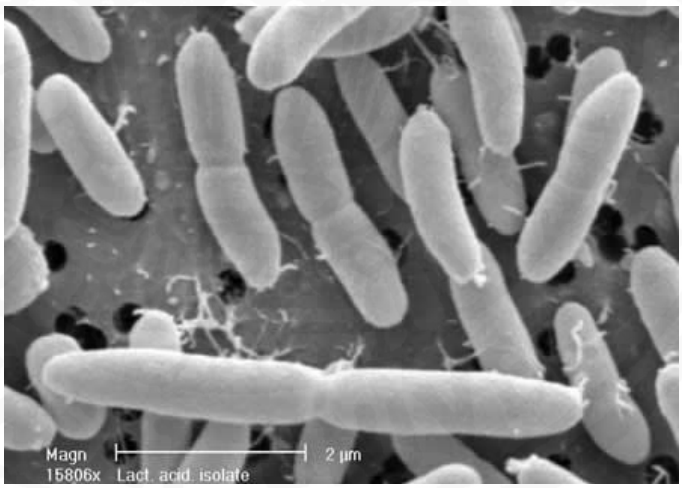
The important microorganisms used in starter cultures for sausage production

Microorganism	**Species**	**Characteristics**	**References**
LAB	*L. plantarum* *L. pentosus* *L. sakei* *L. curvatus*	acid production	Hugas et al., 1993 Rebecchi et al., 1998 Papmanoli et al., 2003 Visessanguan et al., 2006
	Pediococcus acidilactici *P. pentosaceus*	acid production (fast fermenting)	Vural, 1998 Rebecchi et al., 1998
Curing Bacteria (color and flavor forming)	*Kocuria varians (Micrococcus)*	color and flavor	Arihara et al., 1993
	Staphylococcus xylosus *S. carnosus*	color and flavor	Olesen et al., 2004 Stahnke et al., 2002
Yeasts	*Debaryomeces hansenii*	flavor	Durá et al., 2004
	Candida famata	flavor	Olesen and Stahnke, 2000
Molds	*Penicillium nalgiovense* *P. chrysogenum*	color and flavor	López-Díaz et al., 2001 Sunesen and Stahnke, 2003

2.2.1 Lactic acid bacteria used in fermentation of meat

Lactobacilli are gram positive and vary in morphology from long, slender rods, which frequently form chains as shown in Figure 2.1. Lactic acid bacteria (LAB) are generally catalase negative and non-spore forming. The genera *Lactobacillus, Leuconostoc, Pediococcus and Streptococcus* are important members of this group. The growth is optimum at pH 5.5-5.8 and the organisms have complex nutritional requirements for amino acids, peptides, nucleotide bases, vitamins, minerals, fatty acids and carbohydrates (Hutkins, 2006).

Figure 2.1
Morphology of Lactobacilli
(Retrieved January 20, 2010, from http://www.musee-afrappier.qc.ca)



LAB are used in the food industry for several reasons. They play a central role in fermentation process. They ferment various carbohydrates into lactic acid which causes a pH reduction of raw material. It is so called acidification process which is one of the most desirable side-effects of their growth. The pH may drop to as low as 4.0, which is enough to inhibit the growth of most microorganisms including the most common human pathogens, thus allowing prolongation of foods shelf life. The acidity also changes the texture of the foods due to precipitation of some proteins, and the biochemical conversions involving in microorganism growth and the flavor

enhancement. The fermentation or the bacterial growth is self-limiting due to the sensitivity of lactic acid bacteria to such acidic pH. The presence of lactic acid, produced during the lactic acid fermentation is responsible for the sour taste and for the improved microbiological stability and safety of the food (Caplice and Fitzgerald, 1999; Leroy and De Vuyst, 2004).

The improvement of fermented sausages can be achieved by the use of LAB starter culture. Microorganisms belonging to the lactobacillus group, i.e. *L. plantarum*, *L. sakei*, *L. curvatus*, and *L. pentosus* have been employed in meat fermentation (Ammor et al., 2005; Visessanguan et al., 2006; Zdolec et al., 2008). The performance of *L. plantarum* and *L. pentosus* as meat starter cultures for manufacture of the Scandinavian-type fermented sausage was evaluated. The sausages inoculated with *L. plantarum* and *L. pentosus* were rapidly reached final pH of 4.8-5.0 which is desirable to inhibit pathogens and spoilage bacteria, facilitate production of an acidic taste (Klingberg et al., 2005). Vural (1998) used *P. acidilactici* as a starter culture to ferment Turkish semi-dry sausages. The use of this culture significantly reduced the pH, increased lactic acid content and improved the development of the characteristic of sausage such as colour, flavour, firmness of texture and general acceptability.

2.2.2 Carbohydrate metabolism in LAB

Generally, there are two main hexose fermentation pathways that are used to classify LAB genera (Gänzle et al., 2007). Under conditions of excess glucose and limited oxygen, homofermentative LAB catabolize one mole of glucose in glycolysis pathway (the Embden-Meyerhof-Parnas (EMP) pathway) to yield two moles of pyruvate as presented in Figure 2.2. Intracellular redox balance is maintained through the oxidation of NADH, concomitant with pyruvate reduction to lactic acid (Figure 2.3). However, pyruvate can lead to the generation of many other metabolites such as acetic acid, formic acid, ethanol, diacetyl and acetaldehyde. Homofermentative LAB produce more than 85% lactic acid from glucose.

Heterofermentative LAB use the pentose phosphate pathway, alternatively referred to as the pentose phosphoketolase pathway (Figure 2.4). One mole glucose-6-phosphate is initially dehydrogenated to 6-phosphogluconate and subsequently decarboxylated to yield one mole of CO_2. The resulting ribulose-5-phosphate is cleaved into one mole glyceraldehyde 3-phosphate (GAP) and one mole acetyl phosphate. GAP is further metabolized to lactic acid as in homofermentation (Figure 2.3), with the acetyl phosphate reduced to acetic acid and ethanol via acetyl-CoA intermediate (Figure 2.4). Heterofermentative LAB produce only 50% lactic acid and considerable amounts of ethanol, acetic acid and carbon dioxide.

Figure 2.2

Glycolysis pathway

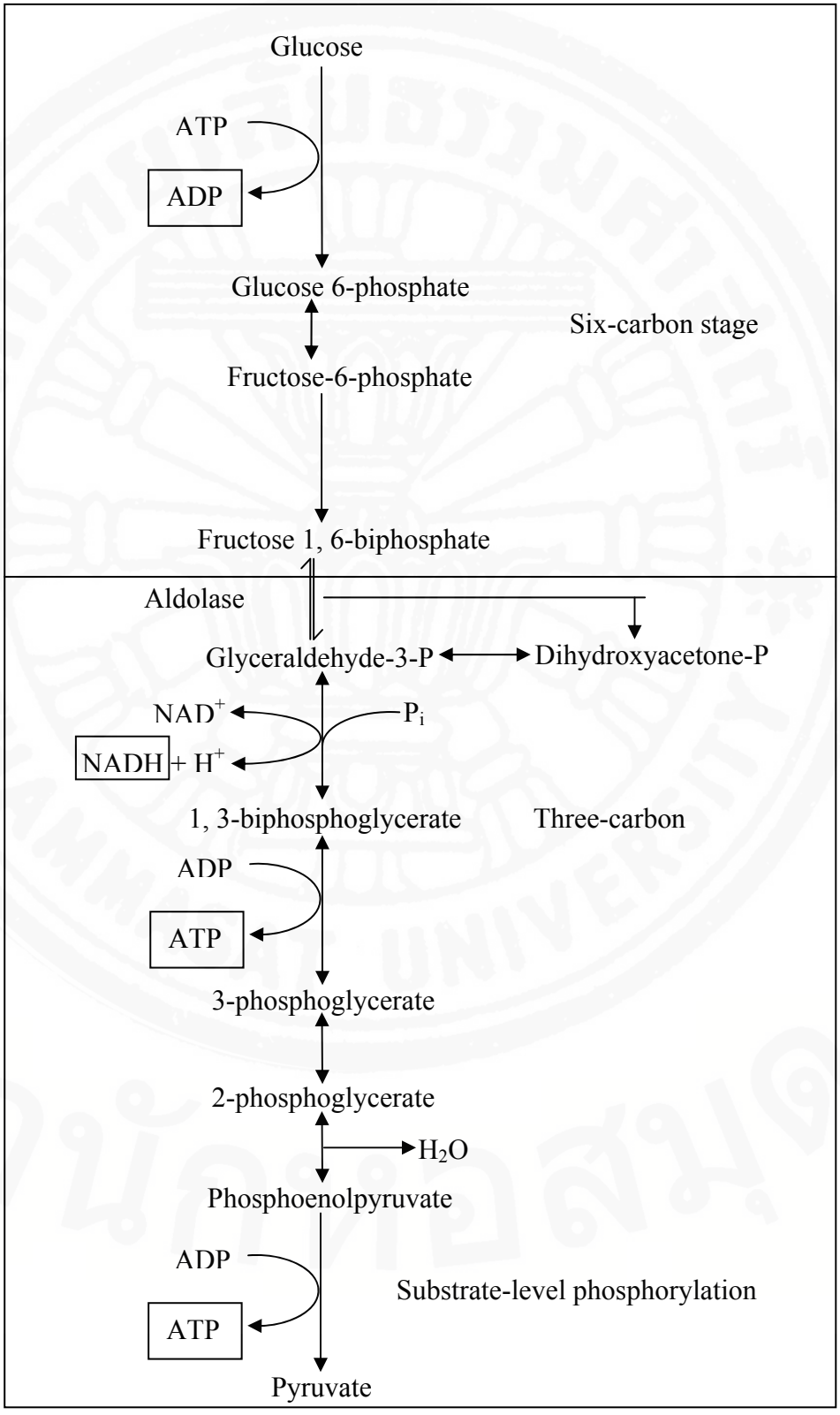


Figure 2.3

Fermentation in Lactic acid bacteria

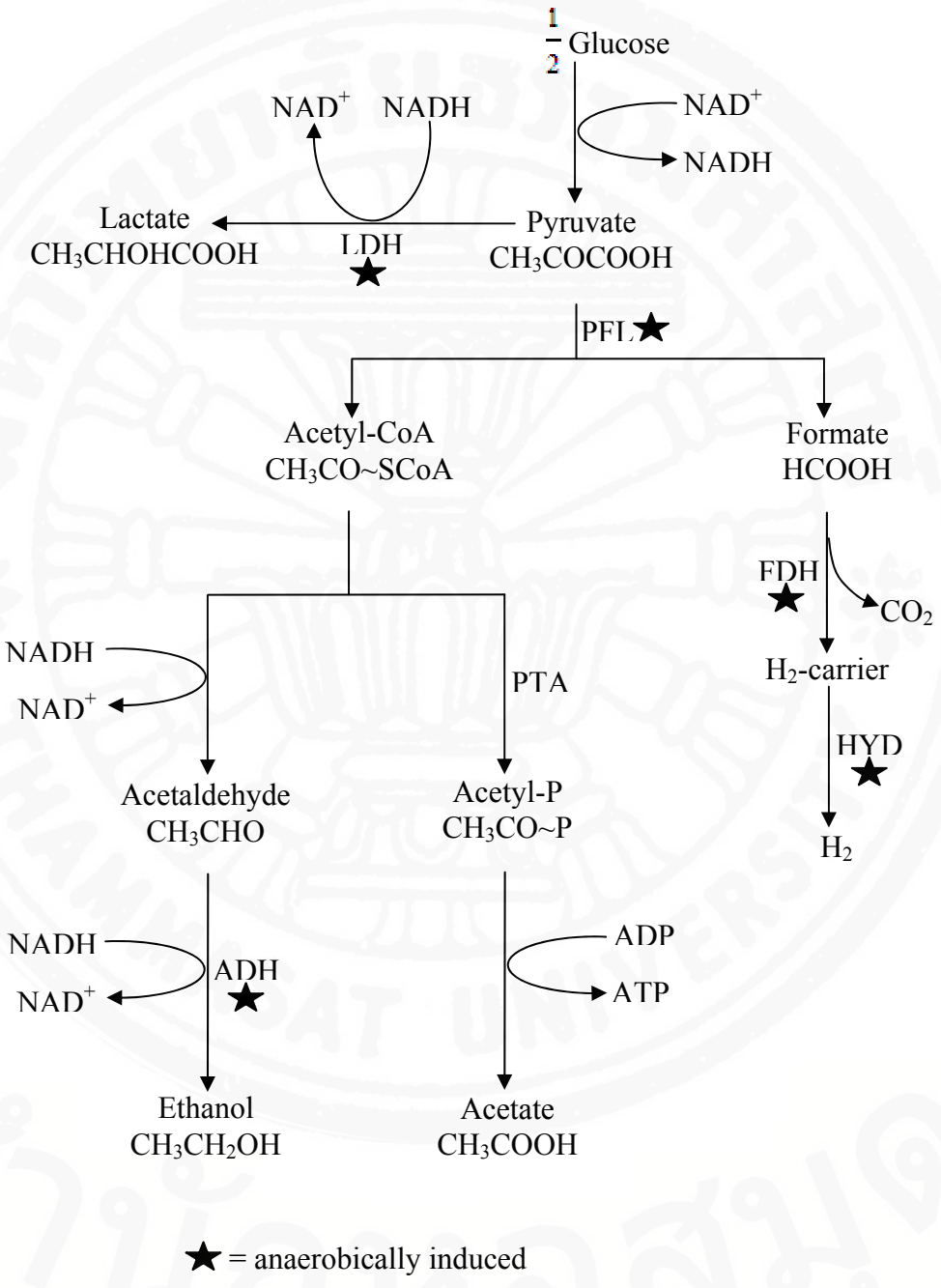
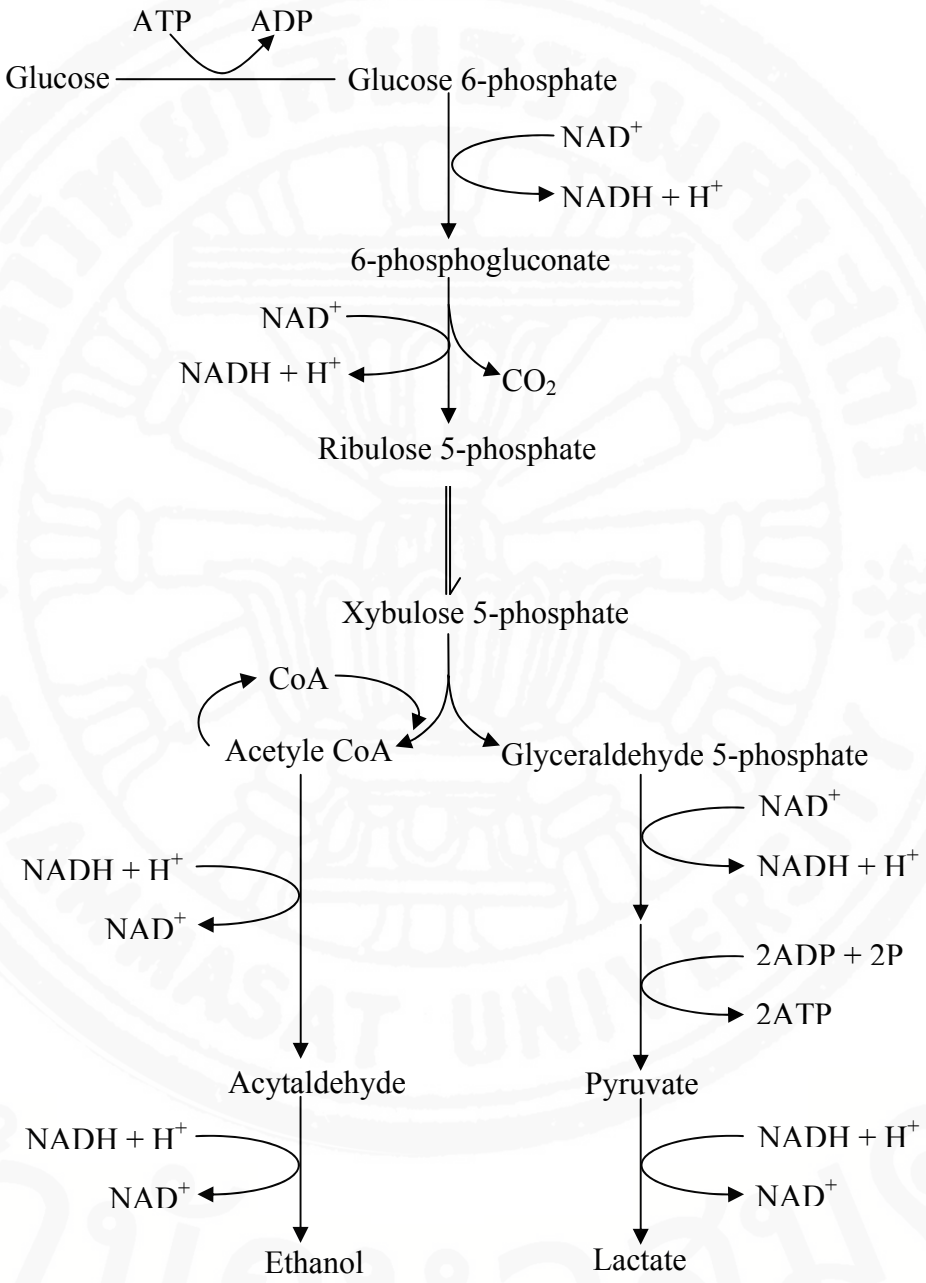


Figure 2.4

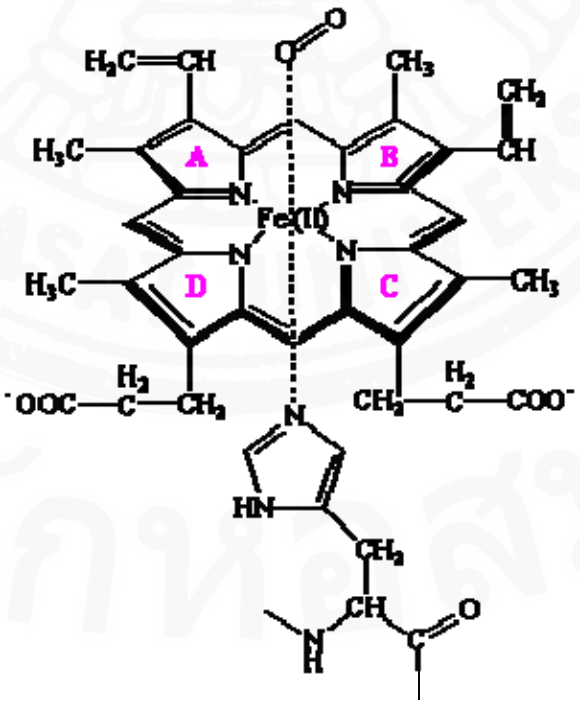
Heterofermentative LAB use the pentose phosphate pathway



2.3 Cured meat pigment

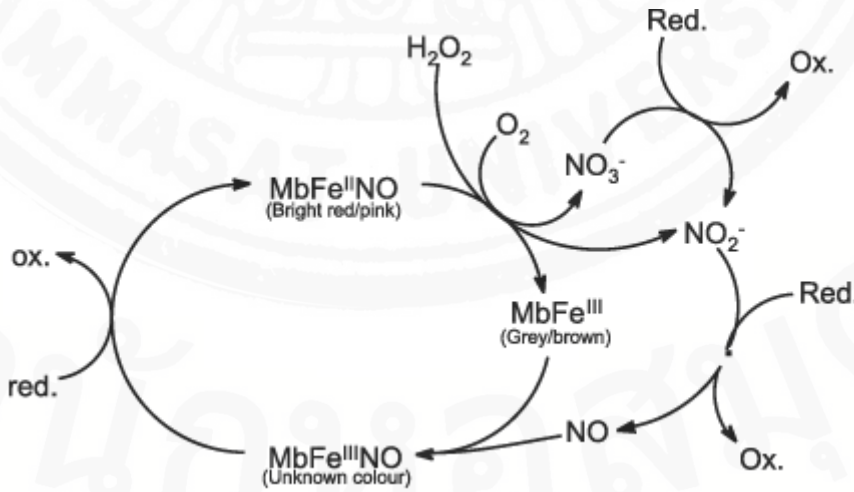
Meat color depends on myoglobin protein, which is part of the sarcoplasmic, or plasma proteins or water soluble proteins. Myoglobin consists of a protein called a globin and the non protein portion as heme ring as shown in Figure 2.5. Within the myoglobin protein structure, there exists a heme structure that normally contains iron (Fe). The oxidation state of the Fe, the soluble or denatured protein structure, and the total amount of myoglobin in the meat are used for the determination of meat color. The Fe centrally located in heme protion of myoglobin has an active site that binds to various compounds, i.e., oxygen, nitric oxide. The compounds bound to myoglobin gives different colors to meat (Voet and Voet, 1995).

Figure 2.5
Structure of myoglobin
(Retrieved January 20., 2010, from http://chemed.chem.purdue.edu)



Colour formation and colour stability are very important quality attributes of meat products. The curing agents are such as nitrate/nitrite, not only served for contribution to flavour, inhibition of rancidity and inhibition of unwanted bacteria, but also used for red/pink colour formation of meat (Møller et al., 2003). The nitric oxide (NO) generated from added nitrate or nitrite is the compound which is responsible for the colour and stability of cured meats. In addition to the oxidation reactions, NO reacts with central Fe (II) in heme of myoglobin to generate nitrosylmyoglobin (MbFe(II)NO) which is an important red pigment colour. In Figure 2.6, the attractive red MbFe(II)NO can be oxidized by H_2O_2 or by oxygen to yield grey/brown metmyoglobin (MbFe(III)) and nitrate or nitrite, respectively. In meat added reducing agent the pools of nitrate/nitrite can be reduced back to NO, which binds to metmyoglobin. This complex (MbFe(III)NO) can undergo reduction, thereby returning to MbFe(II)NO (Møller and Skibsted, 2002).

Figure 2.6
Colour cycle of myoglobin derivatives in cured meat
(Møller and Skibsted, 2002)



Unfortunately, the safety of nitrate and nitrite to human health has been questioned as these may be precursors of carcinogenic nitrosamines from nitrite and secondary amines formed in some cured products. Alternative methods to addition of nitrite have been developed. The effects of microorganisms on meat colour have been determined in recent studies. Several LAB have proved capable of reducing MbFe(III) to MbFe(II), which makes the coordination of NO possible and changes the colour from brown to red. A few strains, such as *Kurthia* and *L. fermentum*, have been reported capable of converting MbFe(III) to the red cured meat pigment MbFe(II)NO (Arihara et al., 1993). Additionally, the bacterial biosynthesis and metabolism of arginine may also produce NO as a side-product in the intermediate reaction (Cunin et al., 1986; Morita et al., 1997). In previous studies of Møller et al. (2003) and Gündoğdu et al. (2006), *L. plantarum, L. fermentum* and *P. acidilactici* had ability to generate NO that could form the basis for production of cured meat products without the use of nitrate/nitrite. Kawahara et al. (2006) found that acceptable shelf stability and colour in cured meat products might be attained by the use of *L. sakei* as a starter culture instead of nitrite. Therefore, NO producing bacterial strains could become an alternative to the usage of nitrate and nitrite in fermented meat product.

2.4 Meat Protein

Muscle or meat protein can be divided into three different fractions on the basis of function and solubility: (1) sarcoplasmic or water-soluble, (2) myofibrillar or salt-soluble and (3) connective tissue or insoluble fraction (Yada, 2004).

The sarcoplasmic fraction consists of those proteins found in the sarcoplasm, or the fluid surrounding the myofibrillar protein. Sarcoplasmic proteins are often referred to as water-soluble proteins because they are commonly extracted with water or low ionic strength salt solutions. This fraction contains various different plasma proteins, i.e., myoglobin, hemoglobin, and flavin nucleotide. There are many enzymes as follows: (i) oxidative enzymes, i.e., cytochromes; (ii) glycolysis enzymes, i.e., glyceraldehydes-3-phosphate dehydrogenase; and (iii) muscle proteinases, i.e., clapains or nutral, serine proteinases and cathepsins or acid, aspartic proteinases. Myoglobin is the one plasma protein that has significant importance in processed

meat because myoglobin gives meat its color. The heme (iron) portion of myoglobin has an active site that binds various compounds. The compounds, i.e., oxygen, nitric oxide, bound to the myoglobin gives different colors to meat (Yada, 2004).

Myofibrillar proteins are also known as contractile proteins. The principle proteins in the myofibrillar fraction include myosin, actin and the combination form of actomysin, which results from contraction of muscle. The myofibrillar or contractile proteins, form the largest structure and bulk of muscle. These proteins form the structure called myofibrils inside the muscle cell and are responsible for the contraction ability of living muscle. The good raw meat materials generally contain the highest level of this protein group. The myofibrillar proteins are distinguished from other meat proteins because they are soluble in high salt solutions and thus, are often called the "salt soluble proteins". These proteins are insoluble in normal meat, but they will absorb enough water in the presence of salt, i.e., at high concentration of ion, the myofibrillar proteins become soluble (Yada, 2004).

2.4.1 Proteolysis

Proteolysis is one of the most important biochemical changes. It influences the development of the texture and flavour compounds due to the degradation of proteins to polypeptides, small peptides, free amino acid and non-protein nitrogen known as flavour precursors (Bruna et al., 2003; Durá et al., 2004; Fadda et al., 2001). The amounts of non-protein nitrogen and free amino acids are increased that verify the degradation of muscle proteins in the fermentation. The tenderization in meat is increased because of the increase of myofibrillar protein fragmentation during ripening of meat product (Aksu et al., 2002; Hughes et al., 2002; Zdolec et al., 2008). DeMasi et al. (1990) reported that during meat fermentation, the activity of endogenous and bacterial proteinases modified the composition of non-protein nitrogen.

Proteolytic activity on the meat proteins has been described for strains of LAB leading to the hypothesis that both endogenous and bacterial peptidases are required for the completion of hydrolysis of oligopeptides. Thus, the starter cultures could be used to accelerate proteolysis. The lactobacillus strains included in this study, *L. sakei*,

L. plantarum, and *L. pentosus*, were also selected on the basis of its high exoproteolytic activity (Fadda et al., 1998). Amongst these selected starter cultures, medium inoculated with *L. plantarum* showed the highest amino acid concentration after 72 h of incubation (Fadda et al., 1998). Meat products inoculated with *Lactobacillus* spp. have shown proteolytic activity on sarcoplasmic and myofibrillar protein. In addition, the ability of *L. sakei*, *L. curvatus* , *L. plantarum* in hydrolysis and amino acid generation derived from muscle proteins, myofibrillar and sarcoplasmic proteins, have been demonstrated *in vitro* (Fadda et al., 1999a and 1999b).

The respective roles of endrogenous and bacterial enzymes in protein degradation during sausage fermentation have been a source of controversy (Verplaetse et al., 1989). However, numerous studies have concluded that endrogenous proteinases, particularly cathepsin D, are primarily responsible for the proteolysis and peptide formation during fermentation, while bacterial proteinases, bound either to the cell wall or to the cell membrane, act on the released oligopeptides during the latter stages (Demeyer et al, 2000; Hierro et al., 1999, Hughes et al., 2002; Molly et al., 1997; Visser, 1993). Besides the necessary amino acid converting enzymes, the peptides generated by muscle proteolysis can also be taken up by bacterial mechanisms that further split them intracellularly into amino acids and may convert these amino acids to aroma components.

2.4.2 Free amino acids

For last proteolytic step, peptides are converted to free amino acid through muscle and microbial aminopeptidases removing single residues sequentially from the N-terminal (DeMasi et al., 1990; Hughes et al., 2002; Roseiro et al., 2008; Toldrá et al., 1993). This action has relevance to the overall acceptance of dried fermented meat product, since free amino acids are the source of specific tastes, odours, and the final flavour characteristics (Flores et al., 1997; Henriksen and Stahnke, 1997). Thus, it is particularly important to determine released free amino acids during fermentation of sausages. Microorganisms produce enzymes to release the amino acids from proteins and special interest is LAB that supply amino acids for optimum growth (Ardö, 2006).

The amino acids such as serine, glycine, threonine, alanine, aspartic acid, and asparagines, could be transformed to pyruvate and then converted to organic acids, mainly lactic acid by LAB metabolism (Adamberg et al., 2006). In the study of Casaburi et al. (2007 and 2008), *L. curvatus* was used as a starter culture for the production of fermented sausages. There was an increase in total amino acids only in sausage inoculated with *L. curvatus* while the concentration of total amino acids was constant in the control sample (uninoculated). Casaburi et al. (2007) reported that at the end of ripening, a greater increase in essential amino acids, mainly valine, leucine, isoleucine, phenylalanine, proline and alanine, was detected in inoculated sausage compared to the control. Similar to the study of Hughes et al. (2002), they found an increase of alanine, valine, leucine, isoleucine, phenylalanine and proline during ripening of inoculated sample. The generation of amino acids is essentially integrated by a cell wall-associated proteinase, peptide transport systems, and a pool of intracellular peptidases (Sanz and Toldrá, 2002). Two general aminopeptidases, PepC and PepN, are commonly found in lactobacilli. These enzymes can hydrolyze basic, aromatic, and hydrophobic amino acid residues from oligopeptides (Kunji et al., 1996). Moreover, the main intracellular peptidases, X-propyl-dipeptidyl peptidase and arginine aminopeptidase of *L. sakei* have been purified and characterized (Sanz and Toldrá, 2001 and 2002). Therefore, the free amino acids were determined during fermentation to evaluate the effect of the starter cultures on proteolysis.

2.5 Flavour formation of fermented sausage

.

The flavour of fermented sausages has been widely studied in recent years. These studies have focused on the mechanism involving flavour formation (Herranz et al., 2005; Ordóñez et al., 1999; Tjener et al., 2004). Flavour is developed from a complex combination of several volatile compounds such as aldehydes, alcohols, ketones, and their acids and non-volatile compounds such as amines, amino acids and small peptides. Proteolysis gives rise to the development of the flavour compounds by proteins degrading to peptides, free amino acid and non-protein nitrogen (Bolumar et al., 2006; Bruna et al., 2003; Demeyer et al, 1995; Durá et al., 2004). Free amino acids are also the origin of other aroma volatile compounds. Some LAB are able to

degrade amino acids further into volatile compounds (Ardö, 2006; Wallace and Fox, 1997). Catabolism of amino acids commonly starts with removal of amino group, which is performed by aminotransferases. LAB are equipped with branched-chain amino acid aminotransferases encoded by *ilvE* that catalyze the conversion of three methyl branched-chain amino acids such as leucine, isoleucine and valine, into methyl-branched keto acids. The keto acids are subsequently converted into the corresponding methyl-branched aldehydes, alcohols or acids (Tjener et al., 2004). Flavor enhancing methyl-branched aldehydes, alcohols and acids derived from branched-chain amino acids (BCAA) leucine, isoleucine and valine are illustrated in Table 2.2. Especially, 2- and 3-methyl-butanal are essential compounds of sausage aroma. Similarly, 2-, 3-methyl-butanoic acid has been reported to be an important contributor to sausage flavour (Berdagué et al., 1993; Montel et al., 1996; Søndergaard and Stahnke, 2002).

Table 2.2

Flavour producing amino acid catabolism in anaerobic environment starting with aminotransferase activity (Ardö, 2006)

Amino acid	**α-keto acid**	**Aldehydes**	**Alcohols**	**Carboxylic acid**
Leucine	α-keto-iso-caproic acid	3-Methyl-butanal	3-Methyl-butanol	3-Methyl-butanoic acid
Isoleucine	α –keto-β-methylvaleric acid	2-Methyl-butanal	2-Methyl-butanol	2-Methyl-butanoic acid
Valine	α-keto-isovaleric acid	2-Methyl-propanal	2-Methyl-propanol	2-Methyl-propanoic acid

Starter cultures such as LAB may be added to fermented sausage mixture to enhance flavour formation (Larrouture et al., 2000; Leory et al., 2006; Molly et al., 1996). The effect of starter cultures on the production of volatile compounds has been studied. Sausages inoculated with *Staphylococcus carnosus* were characterized by a high content of branched aldehydes, i.e., 3-methyl-butanal, 2-methyl-butanal and their corresponding acid, i.e., 3-methyl-butanoic acid, 2-methyl-butanoic acid (Berdagué et al., 1993; Montel et al., 1996).

2.6 Mathematical modelling in fermented sausage

Lactic acid bacteria (LAB), i.e., *Lactobacillus sakei*, *L. plantarum*, *L. pentosus*, *Pediococcus acidilactici* and *P. pentosaceus*, are involved in the production of fermented foods. In the food industry, LAB are added as starter cultures to basic food products, such as milk, meat, vegetables and cereals, with purpose to achieve stable and safe end-products (Caplice and Fitzgerald, 1999). LAB have a potential to produce the antimicrobial lactic acid, and hence preserve the food from spoilage bacteria and foodborne pathogen (Leroy and De Vuyst, 2004). Therefore, they can be used to control the fermentation process by inhibiting the growth of the undesirable microorganisms. In the production of fermented sausage, fermentation of sugars to lactic acid by homofermentative LAB causes the pH to fall to 4.6-5.3. The food environment affected the evolution of the microbial populations and the quality attributes. Recently, mathematical models have been used to predict the relation between the food environment and bacterial funcitionality (Armitage, 1997; Leroy, Degeest and de Vuyst, 2002; Pin and Baranyi, 1998). Particularly the modelling of functional properties of LAB used as starter cultures in food fermentations seems to have application potential. The modelling has been studied about biokinetic of cell growth, sugar consumption, bacteriocin production and lactic acid production (Passos et al., 1993, 1994; Bello and Sanchez-Fuertes, 1995; Doβmann et al., 1996; Gänzle et al., 1998).

The two major types of models used in food technology are empirical and fundamental (McKonald and Sun, 1999). Empirical modelling can be applied to fit the experimental data obtained under specific environmental conditions concerning cell growth, sugar metabolism and the production of functional metabolites. Empirical models are used in food systems to predict the microbial safety or shelf life of products. Mathematical modeling techniques are applied to predict growth of spoilage bacteria and foodborne pathogens and toxin formation under specific conditions (McKonald and Sun, 1999). In addition, the models can be detected critical points production such as temperate and optimized production of metabolic products, i.e., lactic acid, bacteriocin (Zwietering et al., 1990). After these empirical models are obtained, fundamental models can be developed to describe the effect of various variables on the behavior of empirical models. The models are solved numerically by several methods such as Euler integration (Leroy et al., 2002; Leroy and De Vuyst, 2005), Runge-Kutta method (Aggelis et al., 1998; Bâati et al., 2004; Drosinos et al., 2006; Passos et al., 1994) and Adomian decomposition method (Biazar et al., 2003). Finally, mathematical descriptions of microbial growth must be validated by comparison of calculated values with experimental data. Doβmann et al. (1996), the growth behaviour of *L. sakei* and *L. pentosus* was determined in a model system simulating the conditions of fermenting sausages. Minor effects on their growth were observed by varying the concentration of glucose, peptone and sodium nitrite. Temperature and sodium chloride concentration were found to have more effect on their growth.

Bâati et al. (2004) presented an unstructured kinetic model to describe the growth of *L. acidophilus*, the consumption of glucose and the lactic acid production in discontinuous batch carried out at various growth temperatures. The kinetic parameters showed a good correlation between the experimental values and those given by the model. The result indicated that the growth limitation was due to the exhaustion of glucose. The simulation results confimed that the coupling between the cell growth and the lactic acid production observed under 37 °C was less significant for 30 °C and 26 °C.

In the study of Passos et al. (1993), an unstructured model was developed to describe bacterial growth, substrate utilization and lactic acid production by *L. plantarum* in cucumber juice fermentations. A Monod's model and kinetic model proposed by Luedeking and Piret (1958) were used to account for the effect of substrate limitation on the specific growth rate and lactic acid production rate, respectively. It was found that lactic acid production is a function of cell division and biomass concentration. For the cell growth, the model presented good agreement with the experimental results during exponential growth, but suggested values consistently lower than those observed after growth ceased. When the value of $[H^+]_{max}$ was changed ±20%, variations on the overall fermentation were observed for lactic acid and total cell concentration. Total lactic acid concentrations of 81 and 110.5 mM were predicted from maximum cell concentrations of 0.277 and 0.393 g/liter, respectively. In simulation, growth ceased primarily because of hydrogen ion inhibition when the initial pH of the juice was not adjusted to 4.76. At the initial pH of 4.76, the growth ceased due to sugar limitation. Thus, growth termination may be due to either acid inhibition or substrate limitation.

Similar to the study of Fu and Mathews (1999), the batch kinetic behavior of *L. plantarum* in the fermentation of whey lactose was determined. The effects of pH and substrate concentration on cell growth and lactic acid production were taken into account in a Monod's model. The experimental data for substrate concentration was predicted well by the model with a single set of parameter equations over the range of pH specified. The relationship between lactose consumption and lactic acid production was indicated that the substrate consumption at the beginning of the fermentation under a low pH value was mainly used for cell growth and maintenance, resulting in a lower product yield. The cell yield coefficients might not reflect the exact amount of substrate that was not converted to biomass. It was due to the whey lactose used in the fermentation contained trypticase peptone and yeast extract. These were proteins, vitamins and other nutrients that were used for cell growth by *L. plantarum*. In uncontrolled pH experiment, initial lactose concentration affected the lag phase of cell growth, but the stationary phase and death phase were unaffected. This study implied that the substrate inhibition in lactose fermentation by *L. plantarum* without pH control was negligible.

Moreover, Messens et al., (2003) chose a modification of the logistic growth to model the cell growth of *L. curvatus*. They investigated the influence of temperature and pH on the growth of *L. curvatus*. The growth rate was maximal around 35 °C and the optimum pH value for growth of *L. curvatus* was 6.0. At higher temperature, the sugars were faster metabolized and the lactic acid yield coefficient was equal to 1.0 g of lactic acid (g glucose l^{-1}). This confirmed the homofermentative character of *L. curvatus*.

In general, the available models have been validated by experimental data derived after growth of various microorganisms in systems of pure cultures (synthetic liquid media or food extracts) under specific conditions. Since foods such as meat are complicated systems, the validation of models with data directly from food system in their natural state has been recommended (Whiting and Masana, 1994). The development of a structured kinetic model was capable of predicting the microbial responses during aerobic storage of country-style sausage at 3 and 12 °C (Aggelis et al., 1998). The results indicated that the maximum specific growth rate of LAB was always higher at 12 °C than 3 °C. The model had a better predictive ability in batches stored at 12 °C. The model gave unique solution, i.e. the optimization process always converges to constant predicted values for a given sausage batch. They described that the structured model could be applicable to every sausage batch manufactured and stored under the same condition. Moreover, Bello and Sanchez-Fuertes (1995) evaluated the growth of starter culture *Lactobacillus* in dry fermented sausages during the curing process under natural climatic condition and in a controlled drying chamber. The application of the mathematical model could explain the four phases which characterized the behavior of *Lactobacillus*; (a) lag phase, (b) exponential growth, (c) stationary phase, (d) death phase. However, several modeling studies (Aggelis et al., 1998; Houtsma et al., 1996; Walls and Scott, 1996; Whiting and Masana, 1994) have been suggested to describe the growth of microorganisms accompanied by sugar utilization. In meat product, there are proteins which can be consumed by microorganisms.

This study develops an unstructured kinetic model that is capable of predicting the growth of LAB in Thai fermented sausages under two selected batches; (a) without starter culture (control), (b) with starter culture, *P. acidilactici*. The kinetic equations of LAB growth, glucose and protein utilizations, lactic acid, formic acid and non-protein nitrogen productions were investigated.