CHAPTER II

THEORY AND LITERATURE SURVEY

2.1 Nisin

Nisin, one of the food preservative bacteriocins, is a polypeptide with 34 amino acid residues and 5 thio-ether bonds. The molecular weight of nisin is 3,500 daltons. It contains some uncommon amino acids such as lanthionine, methyllanthionine, dehydroalanine, and dehydro-amino-butyric acid which are shown in Figure 2.1 (Anonymous, 2005). Nisin is produced by *Lactobacillus lactis* subsp. *lactis.* It has been shown that nisin has an ability to inhibit gram-positive bacteria which can cause foods putrefaction such as *Clostridium botulinum, Bacillus cereus, Listeria monocytogenes,* and *Bacillus stearoacidophiles* – especially *Bacillus alcalophilus* and spore of bacteria which tolerates heat treatment under pasteurization (Nicechem, 2005). Currently, many countries allow the use of nisin as a preservative in many products, for example, dairy products, meat products, aquatic products, canned food, fruit juices, baked goods, fast foods, beverages, cosmetics, medicine, and health care products (Nicechem, 2005). For Quantity limited, nisin must be less than 0.2 g/kg in canned food, less than 0.5 g/kg in meat products and dairy products, and less than 12.5 mg/kg in cheese and butter (Nawawong, 2005). Due to its high stability under acid and heat treatment and it is nontoxic for consumers, nisin is the only bacteriocin which is allowed to use as a food ingredient by United States Food and Drug Administration (USFDA). Nisin was awarded the Generally Recognized as Safe (GRAS) designation in the U.S. Federal Register in April, 1998 and is currently recognized as a safe food preservative in over 50 countries (Anonymous, 1996). Moreover, nisin can be incorporated into antimicrobial packaging material by blending nisin with molten polymer and/or dissolving both nisin and polymer in the same solvent before forming the film by extrusion and injection molding.

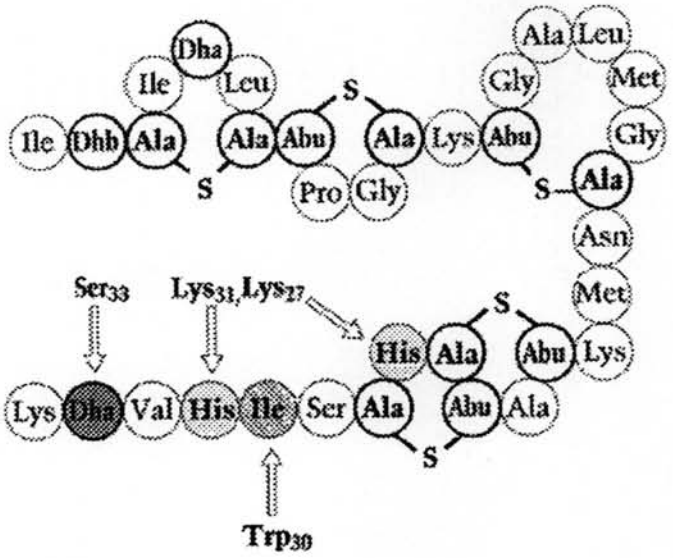

Figure 2.1 Nisin molecule structure.

Source: Sen et al. (1999)

Application of Nisin in Antimicrobial Packaging Material

Padgett et al. (1998) incorporated 0.1 - 6.0 mg of nisin per g of film into biodegradable protein film and determined antimicrobial properties of the films that were formed by heat-pressing and casting methods. Heat-pressing was used to produce films from soy-protein isolate and corn zein while casting was used to produce films from corn zein only. Packaging films with lysozyme or nisin incorporated into the film were tested separately for the inhibition against *Lactobacillus plantarum* NCPO 1752. The researchers found that both cast and heat-pressed films with added lysozyme or nisin formed excellent films and exhibited inhibition of bacterial growth.

Siragusa et al. (1999) incorporated 8 g of nisin in dried milk solids with two hundreds grams of powered low density polyethylene. Films were produced using a 19-mm diameter single screw extruder with a 0.5 inch blown film die. The effect of incorporated nisin on the inhibition of the indicator bacteria *Lactobacillus helveticus* and *Brochothrix thermosphata* on beef carcass surface sections was studied. The results

showed that nisin incorporated films could inhibit both bacteria. Temperature abuse was simulated by shifting inoculated packs from 4 °C (after 2 days) to 12 °C. Within 20 days, it was reported that the *Brochothrix thermosphata* populations on samples wrapped with nisin impregnated film were significantly lower ($p<0.05$) than control vacuum-packaged sample; log 5.8 vs. 7.2 CFU/cm^2, respectively.

Hoffman et al. (2001) studied the inhibition effect of 0.188 mg of nisin, 200 mg of lauric acid (LA), and 27.9 mg of EDTA per film which were incorporated into a corn zein film (10 mL of polymer solution) against *Listeria monocytogenes* and *Salmonella* Enteritidis. The results demonstrated that only films that incorporated nisin and LA could inhibit bacterial growth after 48 hours.

Lee et al. (2004) coated paperboard with antimicrobial film which was vinyl acetate ethylene copolymer incorporating 3% w/w (db) nisin. Paperboard coated only with plain binder was used as control sample. They reported that at 3 and 10 °C, the antimicrobial paperboards retarded microbial growth and lowered the maximum growth levels in both milk and orange juice when compared with control. At 3 °C, the respective lag times of bacteria in milk and yeasts in orange juice were significantly extended by the antimicrobial paperboards coated with nisin.

2.2 Gelatin

Gelatin is produced by partial acid or alkaline hydrolysis of collagen extracted from skin, bones, cartilage, ligaments of animals (Anonymous, 2006). Molecular structure of gelatin produced by hydrolysis is polypeptide with 18 types of amino acids. Table 2.1 shows amino acid composition of gelatin. It contains a large proportion of glycine (almost 1 in 3 residues, arranged every third residue), proline, and 4-hydroxyproline residues (Chaplin, 2006). Gelatin is a translucent brittle solid, colorless or slightly yellow, nearly tasteless and odorless. It contains 9% - 12% humidity and its specific gravity is 1.3 - 1.4. When dissolved in water, soluble gelatin absorbs water to

about 10 times of its dry weight. The gelatin solution is positively charged. Gelatin can form soft gel which has the melting point at 37 °C.

Table 2.1 Gelatin Composition.

Amino acids	%by weight	Amino acids	%by weight
Alanine	11.00	Methionine*	0.90
Arginine	8.80	Phenylalanine	2.20
Aspartic acid	6.70	Proline	16.40
Glutamic acid	11.40	Serine	4.20
Glycine	27.50	Threonine**	2.20
Histidine	0.78	Tyrosine	9.30
Leucine and Isoleucine	5.10	Valine	2.60
Lysine	4.50	Cystine	trace

* Essential amino acid which could be eliminated by oxidation

** Essential amino acid which could be eliminated either by hydrolyzing or at the gelatin process

Source: Asakong and Bunlausak (2002)

2.2.1 Structure of Gelatin

As a partial hydrolysis product of collagen (Figure 2.2), the structure of gelatin is similar to collagen but it has shorter polypeptide chains (Figure 2.3). Transforming collagen into gelatin could be done by using acid, alkaline, enzyme, or temperature controlling resulting in breaking of collagen molecule chains and reforming into gelatin which has a very wide range of molecular weight; from 2,000 up to hundreds thousands atoms (Kittisopaporn et al., 1990). The typical gelatin manufacturing process can be classified into 2 methods: acid process (for type A gelatin) and liming process (for type B gelatin).

$$C_{102}H_{149}N_{31}O_{38} + H_2O \longrightarrow C_{102}H_{151}N_{31}O_{39}$$

[Collagen] [Gelatin]

Figure 2.2 Hydrolysis of collagen to gelatin.

Source: Shreve (1967)

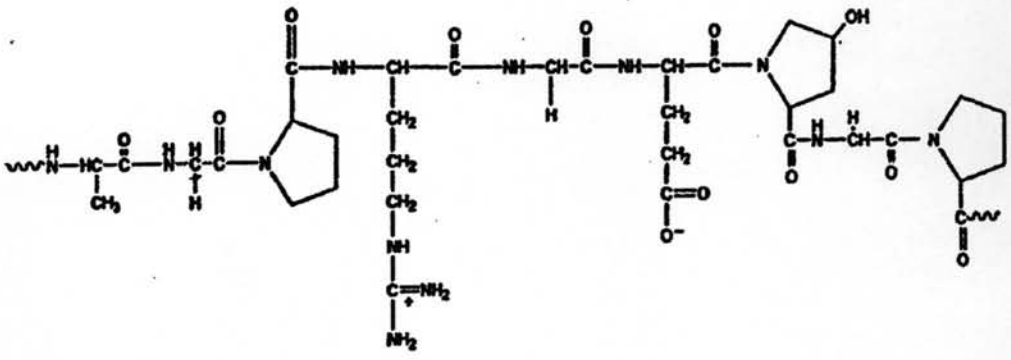

Figure 2.3 The general structure of gelatin.

Source: Chaplin (2006)

Gelatin is a natural biological polymer which is biodegradable and biologically compatible. It is safe for human consumption. When gelatin is heated in water at 32 °C, it forms viscous solution and solidifies to elastic gel when cooled to below 32 °C. In the food industry, gelatin is used as an ingredient in food products such as dessert, marshmallow, jam, jelly and is used for packaging, protective stability properties of baked product and ice-cream. Gelatin is also used in pharmaceutical industry for the manufacture of soft and hard capsules. The useful functions of gelatin in photographic film manufacture are a protective colloidal properties during the precipitation and chemical ripening of silver halide crystals, setting and film-forming properties during coating, and swelling properties during processing of exposed film or

paper. For cosmetic industry, gelatin is blended with cream for increasing surface tension. Besides, gelatin is used to produce PVC (Polyvinyl chloride) (Asakong and Bunlausak, 2002).

2.2.2 Gelatin for Packaging Material Preparation

Gill and Holley (2002) studied the effect of antimicrobial agents incorporated gelatin coating on cooked ham and bologna. Cooked 10 g disks of ham and bologna sausage received one of three treatments: (1) no coating (control), (2) coating with 0.2 g of 7% w/v gelatin gel (gel-control), or (3) coating with 0.2 g of 7% w/v gelatin gel containing 25.5 g/L of lysozyme-nisin (1: 3) plus 25.5 g/L of EDTA (gel-treated). The samples were then incubated with one of six test organisms: *Brochothrix thermosphacta, Escherichia coli* O157:H7, *Lactobacillus sakei, Leuconostoc mesenteroides, Listeria monocytogenes,* and *Salmonella* Typhimurium. Inoculated samples were vacuum packed and stored at 8 $^{\circ}$C for 4 weeks. The third treatment had an immediate bactericidal effect up to 4 log CFU/cm^2 on the four gram-positive organisms tested and inhibited the growth of *Salmonella* Typhimurium during 4 weeks storage. The numbers of *E. coli* O157:H7 on ham were reduced by 2 log CFU/cm^2 following the second and the third treatments. No effect was observed on the growth of *E. coli* O157:H7 on bologna.

Asakong and Bunlausak (2002) studied the possibility of preparing gelatin film which was used for casing sausages. Gelatin was modified by 5% - 40% w/w stearic acid (pH 4.5) at 60 $^{\circ}$C for 2 hours. They reported that modified gelatin film had less water absorption, tensile strength, elongation, and time to dry, compared to unmodified gelatin film. Thus, modified film with 5% w/w stearic acid was used to produce sausage casing.

2.3 Electrostatic Spinning

Electrostatic spinning technique is a simple and versatile method for fabricating nanofibers. Nanofiber is a fine fiber having diameter from about 10 nanometers up to less than one micrometer (Hongrodjanawiwat et al., 2004). The advantage of nanofiber is its high surface area to volume ratio. It has been reported by Mit-uppatham et al. (2004) that nanofiber could possess up to 1,000 to 10,000 times the surface area to volume ratio of a microfiber. Therefore, nanofiber has a tendency to be more popular for a number of applications in the future.

The electrospinning process has been developed for over 70 years since Formhals was issued his patent on electrospinning of polymer fibers in the United States (US Patent 1-975-504) in 1934. After that, hundreds of patents were issued on electrospinning, regarding equipment designs, production, and applications of nanofibers by other researchers (Mit-uppatham et al., 2004). The equipment consists of three main parts, as shown in Figure 2.4, which are:

1. High-voltage power supply which could be adjusted an electrical potential between 3 to 30 kV. Electrical power supply should be less than milliampere to avoid danger.

2. Glass syringe for sample loading which has a hole for polymer to flow out.

3. Metal collector such as metal plate or sieve or rotating metal drum. Polymer sample could be in the form of solution or melt. Electrostatic spinning could be either sensible horizontal (by placing injection tube with a small angle to the ground to avoid spilling out of the sample) or horizontal line. Anyhow, this depends on viscosity of polymer sample.

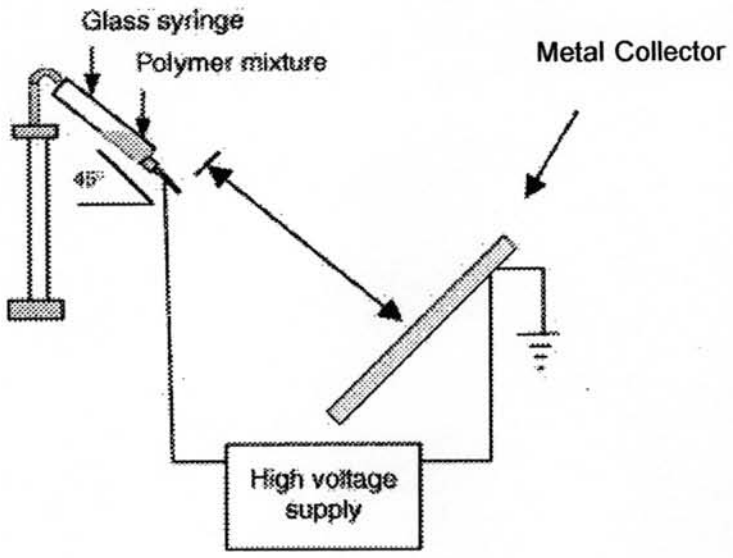

Figure 2.4 Electrostatic spinning apparatus set-up.

Source: Artphop et al. (2006)

Comparing electrospinning with other spinning techniques which can produce highly fine fibers, it can be clearly seen that electrostatic spinning technique has greater advantages such as less complexity, the required equipments are not expensive and use small amount of polymer solution. Although, the speed of fiber spinning is very high, the diameters of fibers are very small. Hence, it takes a long time in order to get enough amount of fiber.

The mechanism of nanofibers production by electrostatic spinning begins from providing increasing high voltage to polymer solutions or melts until hemispherical surface of the solution at the tip of the capillary tube elongates to form a conical shape known as the Taylor cone. When the electric field reaches a critical value at which the repulsive electric force overcomes the surface tension force, a charged jet of the solution is ejected from the tip of the Taylor cone. The jet extends in a straight line for a certain distance and then bends and follows a looping and spiraling path like a bending instability to collecting metal screen as shown in Figure 2.5. The electrical forces elongate the jet thousands or even millions of times and the jet becomes very thin. Ultimately, the solvent evaporates, leaving a charged polymer fiber behind which

lays itself randomly on a collecting metal screen for electrostatic spinning from polymer solutions or the melt solidifies by cooling down for electrostatic spinning from polymer melts (Fong and Reneker, 2001).

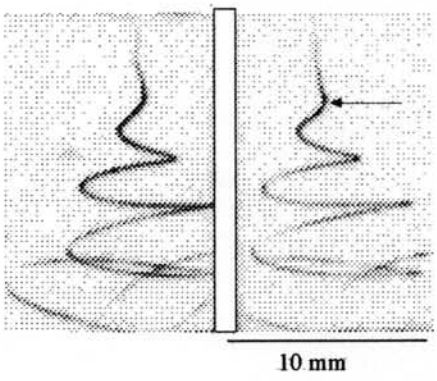

Figure 2.5 Bending instability.
Source: Darrell (2003)

In general, the resulting, very long, nanofiber collects on an electrically grounded metal sheet, a winder or some other object, often in the form of a non-woven fabric. The electrospinning process makes fibers with diameters in the range of one or two orders of magnitude smaller than those of conventional textile. Nanofibers are being used in biomedical applications including wound dressings and drug delivery system. Nanofiber offers advantages for the application of pesticides to plants, protective clothing and filtration (Mit-uppatham et al., 2004).

2.3.1 Parameters Effect of Electrostatic Spinning Process (Mit-uppatham et al., 2004)

There are three parameters affecting the electrospinning process which are:

1. Characteristics of polymer melts or polymer solutions such as viscosity, surface tension, electrical conductivity (related to concentration of free charges in polymer current).

2. Process parameters such as electrical potential, initial flow rate and distance between the capillary tube and the collecting screen.

3. Ambient parameters such as temperature, humidity, pressure, type and concentration of free charges in air, and electric field or magnetic field of the chamber.

These parameters exert an effect on nanofibers size and shape. The most important parameter that defines nanofibers size is initial viscosity of polymer melt or polymer solution. The viscosity is directly related to temperature polymer molecular weight and concentration for polymer solution. An increase in solution viscosity causes an increase in nanofiber diameter. Besides, the increase of electrical potential between the tube tip and collecting metal screen results in nanofibers with bigger diameter. However, there are many studies that reported a decrease in diameter of nanofiber with increasing electrical field strength.

The disadvantage of nanofiber which is produced from electrostatic spinning process is bead formation on fibers. Generally, beads occur from low viscosity solution. This is because the surface tension of low viscosity polymer solution overcomes the electrical force at the surface of the solution or polymer melt and viscosity. Increasing polymer solution concentration, decreasing its surface tension by adding solvent or chemical substance or adding salt (such as sodium chloride, lithium chloride) which can be decompounded into free charges in polymer solution can reduce bead formation. This is because free charges, which have an effect on the impact force between charges, exert more effect than surface tension.

2.3.2 Electrostatic Spinning of Biopolymer

Choktaweesap (2004) studied the preparation of electrospun gelatin nanofibers. Gelatin solutions were prepared in single (acetic acid) and various solvents (acetic acid/dimethyl sulfoxide; Ac/DMSO, acetic acid/ethylene glycol; Ac/EG, acetic acid/formamide; Ac/F, and acetic acid/trifluoroethanol; Ac/TFE) prior to electrospinning. When gelatin concentration is increased from 15% w/v to 29% w/v in the single solvent system, it was found that the average diameter of the as-spun fibers was increased from about 214 nm to about 840 nm. For the mixed solvent systems, solvent properties affected appearance of the electrospun webs and diameter of gelatin fibers. Particularly, for Ac/DMSO solvent at various ratios ranging from 97: 3 to 91: 9, the obtained fibers were bead-free and their diameters were increased with an increase in the amount of DMSO in the mixed solvent.

Huang et al. (2004) investigated electrospinning of gelatin and the mass concentration-mechanical property relationship of the resulting nanofiber membranes. 2, 2, 2-Trifluoroethanol (TFE) was used as the solvent. The resulting solution with a mass concentration between 5% and 12.5% was successfully electrospun into 100 nm to 340 nm nanofibers. Lower or higher mass concentration was inapplicable in electrospinning at ambient conditions. They reported that the highest mechanical behavior did not occur to the nanofibrous membrane electrospun from the lowest or the highest mass concentration solution. Instead, the nanofiber material that had the finest fiber structure and no beads on surface obtained from the 7.5% mass concentration exhibited the highest tensile modulus and ultimate tensile strength, which were 40% and 60%, respectively, which were greater than those produced from the remaining mass concentration, i.e. 5%, 10%, and 12.5% solutions.

Ki et al. (2005) dissolved gelatin in formic acid and gelatin nanofiber was successfully prepared by the electrospinning using gelatin-formic acid dope solution. Gelatin nanofiber was successfully produced from 7% to 12% w/w gelatin solution. Even though the viscosity dropped markedly after 5 hours, the spinability and morphology of gelatin nanofiber were not affected. The parameters, such as electrical

field, spinning distance, and concentration of dope solution, were probed for their effects on electrospinnability and morphology of gelatin nanofiber web. The structure transformation, from a helical (α-helix and triple-helix) to random coil conformation, might occur when formic acid was used for the dissolution of gelatin in electrospinning.