CHAPTER II
REVIEW OF THE RELATED LITERATURE

A. Previous Research

The researcher will describe some researches which are relevant to this research to make this thesis arrangements easier and to avoid repeating the same research. The research is inspired from Thesis of:

Edi Riswandi (2001) the student of technology faculty, Institute of Agriculture Bogor with thesis entitled "Pengaruh Proses Perendaman Dalam Larutan Enzim Papain Dan Penyimpanan Terhadap Sifat Mutu Daging Kambing Betina Tua". The objective of his thesis are to learn about the influence of submersion process of old goat meat in variety of papain enzyme solutions and the influence of storage about quality of meat. The quality is measured by Physical analysis (pH, water holding capacity, cooking loss, tenderness) and Organoleptic test (appearance, smell, flavour, tenderness, and juiciness).

The similarities between this research and Edi's are in the technique. Both of them focuses on proteolytic enzyme or protease is used to increse meat quality. Both of them use physical analysis and organoleptic test.

The differences from this thesis are in the subject and kind of proteolytis enzyme that used. This thesis uses old goat meat and papain enzyme, but this research uses local beef and bromelain enzyme.

Chris R. Calkins, Ph.D. and Gary Sullivan (2007) entitled "Adding Enzymes to Improve Beef Tenderness", journal of National Cattlemen's beef association, (University of Nebraska). This experiment was conducted at the University of Nebraska- Lincoln to compare all of the Generally Recognized as Safe (GRAS) enzymes and evaluate the activity and tenderizing effect of each in muscles of different connective tissue contents. The study examined at the degradation of the myofibrillar and connective tissue components of muscle. Just five of the many exogenous enzymes (Papain, Bromelain, ficin, Bacillus Protease, and Aspartic protease) that have been studied have been classified as 'Generally Recognized as Safe' (GRAS) by USDA's Food Safety Inspection Service (FSIS) and come from varying plant, bacterial, and fungal.

The similarities between this research and Chris research is in the proteolytic enzyme. In Chris research one of the kind of the GRAZ enzyme is used bromelain enzyme to know tenderizing effect, and this research use bromelain enzyme too.

The differences can be explained as this experiment was conducted at the University of Nebraska- Lincoln to compare all of the GRAS enzymes and evaluate the activity and tenderizing effect of each in muscles of different connective tissue contents. While this research uses bromelain enzyme from pineapple's peel to know the influence this enzyme in meat quality.

Based on the previous studies, then the resercher is interested to compose research based on the title "The influence of waste of pineapple's peel (*Ananas Comosus (L) Merr*) as source of Bromelain enzyme to increase meat (local beef) quality".

B. Theoritical Framework

1. Meat Quality

Meat is defined as the flesh of animals used as food.[1] The muscle of animal is changed to be meat after slaughtering, because the physiology function has stopped. Muscle is main component of meat.[2]

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[1] Lawrie, R. A, *Meat Science*, (Switzerland: woodhead Publishing, 1998), sixth edition, p. 1

[2] Soeparno, *Ilmu dan Teknologi Daging*, (Yogyakarta: Gadjah mada University Press, 2009), p. 2

A meat is arranged of four cell tissues, there are skin tissue, held tissue, fat tissue, and meat tissue. According to Muchtadi and Sugiyono (1992), the main physic component of carcass is sceletal meat tissue. The chemistry component of meat are water (65-80%), Protein (16-22%), fat (1,3-13%), carbohydrate (0,5-1,3%), and mineral (1%).[3] Relatively, nutrient content of meat from various livestock and fish are different, but every 100 gram of meat can fulfill nutrient requirement of a adult each day about 10% calorie, 50% protein, 35% Fe.[4]

Meat quality is defined by the compositional quality (lean to fat ratio) and palatability (appearance, juiciness, tenderness, and flavor) of meat. The appearance of meat deals with the visual identification of quality meat based on color, marbling, and water holding capacity. The meat should have a normal color that is uniform throughout. In addition, it should have marbling throughout the cut. Marbling can be defined as small streaks of fat dispersed within the meat; it is an indication of tenderness and juiciness as well as flavor. The water holding capacity can be determined by looking in the meat package. If excess water is seen at the bottom of the package, this may be an indication of decreased juiciness and increased risk of drying.

Juiciness depends on the amount of water retained in the cooked meat product. It increases flavor, helps soften meat, and stimulates saliva production in the mouth. Juiciness is determined by water retention and lipid content. Marbling and fat around the edges of the cut of meat also help to keep the water in. Water losses can result from evaporation or dripping. The use of proper cooking methods is another way of increasing meat juiciness.

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[3] Edi Riswandi, "Pengaruh Proses Perendaman Dalam Larutan Enzim Papain dan Penyimpanan Terhadap Sifat Mutu Daging Kambing Betina Tua", thesis, (Bogor: Technology faculty, Institute of Agriculture bogor, 2001), p. 4-5

[4] Soeparno, *Ilmu dan Teknologi Daging*, (Yogyakarta: Gadjah mada University Press, 2009), p. 3

Flavor is another factor in meat quality. Meat flavor can be described as salty, sweet, bitter, etc. Meat flavor can be affected by age, water retention during cooking, and fat within the muscle.[5]

There are some factors affecting meat quality, i.e. pH, water holding capacity, cooking loss, and tenderness. Each of them is put in the following explanation.

a. pH

pH is a measure of the acid or alkaline level of the meat. Just might measure the acidity of the soil for optimum growth and productivity, Meat Standard Australia (MSA) measures the acid level of the meat to ensure eating quality. MSA graders measure the pH of the carcase at grading using a pH meter. This measurement is known as the ultimate pH. pH can be measured from 0, which is very strong acid to 14, which is very strong alkaline.[6]

According to Byrne *et. al.*, Meat pH, as affected by post-mortem glycolysis in muscle tissue, has a profound influence on meat quality since it determines traits responsible for the processing suitability and eating attributes of meat [van Laack *et al*. 2001]. This is also the simplest parameter characterizing the course of post-mortem changes in muscles. Thus, the determination of pH is applied in practice to detect meat quality deviations.[7]

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[5] Carrie Eichenberger, *et.al.*, "Sheep Processing: Start to Finish", in http://ag.ansc.purdue.edu/sheep/ansc442/Semprojs/2004/process/index.htm, accessed on Desember, 12nd 2012

[6] Meat and livestock Australia, "The Effect of pH on Beef Eating Quality", in http://www.shorthornbeef.com.au/assets/files/PH_and_Beef_eating_quality.pdf, accessed on January, 12nd 2013

[7] Tomasz Daszkiewicz, *et.al.*, "Quality of Meat From Young Bulls in Relation to Its Ultimate pH Value", Journal of Animal Science (Poland: Department of Commodity Science of Animal Raw Materials, University of Warmia and Mazury in Olsztyn, 2009), p.293

Based on statement above, pH is one of the simplest pharameter quality of meat. Decreasing and increasing pH of meat is affected by post-mortem glycolysis in muscle tissue. Measuring pH of the carcase uses pH meter. Measuring pH starts from 0, which is very strong acid to 14 which is very strong alkaline.

b. Water Holding Capacity

The water-holding capacity (WHC) of meats is related to the amount of free water released by the meat after physical pressure or force is exerted upon it. The freewater area in square inches is determined by subtracting the meat film area from the total wetted area after measurement with a compensating planimeter. Although many studies have been conducted concerning the effect of various parameters on WHC, such as age and sex of animal, pH, and metal ions[8]

According to Fennema research, 1985, free water is water whose flow from the tissue is unimpeded. Weak surface forces mainly hold this fraction of water in meat. Free water is not readily seen in pre-rigor meat, but can develop as conditions change that allow the entrapped water to move from the structures where it is found.

While Honikel, 2004;Honikel & Kim, 1986 in the Journal entitled *Mechanisms of Water-Holding Capacity of Meat: The Role of Postmortem Biochemical and Structural Changes*, explains that the majority of water in muscle is held either within the myofibrils, between the myofibrils and between the myofibrils and the cell membrane (sarcolemma), between muscle cells and between muscle bundles (groups of muscle cells). Once muscle is harvested the amount of water and location of that water in meat can change

[8] James M. Jay, "Relationship Between Water-Holding Capacity of Meats and Microbial Quality", Journal (USA: Department of Biology, Wayne State University, 1964), p.120

depending on numerous factors related to the tissue itself and how the product is handled.[9]

Water holding capacity is the next pharameter that will be discussed in this research. As researcher knows that water holding capacity is affected by amount of free water released by the meat after physical pressure. Water holding capacity determination can be known using sentrifuge method.

c. Cooking Loss

In the journal entitled *Effect of cooking method on mechanical properties, colour, and structure of beef muscle (M. pectoralis),* 2006, Garcia- Segovia *et. al.* Explains that the cooking process of beef from the above cattle breeds is an important tool for the sensory perception of beef by consumers. Cooking is a process of heating beef at sufficiently high temperatures that denatures proteins and makes it less tough and easy to consume. And according to Shilton et al., 2002, It can be achieved either by boiling or by roasting and in all cases losses occur. Cooking loss, which is one of the meat quality parameters that is often ignored by meat scientists and technologists, refers to the reduction in weight of beef during the cooking process. The major components of cooking losses are thawing, dripping and evaporation. Thawing loss refers to the loss of fluid in beef resulting from the formation of exudates following freezing and thawing. Such losses are lower following a rapid freezing compared with slow freezing. This is because of small crystallization formed by the rapid freezing.

[9] Elisabeth Huff-Lonergan, *et.al.*, “Mechanisms of Water-Holding Capacity of Meat: The Role of Postmortem Biochemical and Structural Changes”, Journal of Meat Science (USA: Department of Animal Science, Iowa State University, 2005), p.195

Dripping is the loss of fluid from beef cuts and water evaporation from the shrinkage of muscle proteins (actin and myosin).[10]

Thirtd pharameter of meat quality is cooking loss. In short, cooking loss will occur when the meat is boiling or roasting.

d. Tenderness

Tenderness is one of or the most discussed features in meat. It is a real challenge for the scientific community and for the meat industry to achieve products with standardized and guaranteed tenderness, since these characteristics are exactly what consumers want in a meat product. The United States meat industry has identified solving the problem of inconsistent meat tenderness as a top priority. This requires a detailed understanding of the processes that affect meat tenderness and, perhaps more importantly, the utilization of such information by the meat industry.[11]

Tenderness of meat is affected by both pre slaughter and post slaughter factors. Pre slaughter factors include species, breed, age, sex, feeding and management, genetic influence and stress conditions.

Post slaughter factors that influence meat tenderness include, postmortem glycolysis, postmortem shortening, conditioning, processing and cooking methods. The lowering of pH in muscle due to accumulation lactic acid is one of the most significant postmortem changes that occur due to postmortem glycolysis. Several researchers have reported that the rate of postmortem

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[10] N. Jama, *et.al.*, "Cooking loss components of beef from Nguni, Bonsmara and Angus steers", Journal of Agricultural Research (Replubic of South Africa: Department of Livestock and Pasture Science, University of Fort Hare, 2008), p.416-417

[11] Luciano, *et.al.*, "Biochemical Aspects of Meat Tenderness:A Brief Review", in http://www.uco.es/organiza/servicios/publica/az/php/img/web/25_13_47_889BiochemicalLuciano.pdf, accessed on January, 12nd 2013

glycolysis is an important determinant of meat tenderness. Devine *et al.* (2006) explains that stated that low ultimate pH was necessary to obtain optimum tenderness. Postmortem shortening due to permanent actomyosin bond formation during the development of rigormortis contributes to the muscle stiffening. The meat obtained from such stiff muscle is considered as tough meat. It has long been recognized that the tenderness of meat increases when it is conditioned and for this purpose venison is regularly aged.

In order to improve the tenderness of meat, a number of tenderizing methods have been tried as antemortem or postmortem treatments. Antemortem treatments include feeding of electrolytes, use of enzymes etc.[12]

With this information, the writer can conclude that tenderness is the most important pharameter to know meat quality. The United State meat industry has identified problem solving of meat tenderness. To improve the tenderness, some researchers uses enzyme from various plants, bacteria, and fungal like Papain, Ficin, Bacillus protease, Asparctic protease, and Bromelain. For this research, the writer will use bromelain enzyme to improve tenderness of local beef.

2. Pineapple (*Ananas Comosus (L) Merr*)

The pineapple (*Ananas Comosus (L) Merr*) is a non climacteric fruit and is a terrestrial member of the diverse family Bromeliaceae. Each pineapple plant gives a single fruit before producing suckers which could be used for future planting. The plant is perennial herb 50-100 cm high. It has narrow, tapering, pointed leaves up to 100 cm long arranged in spiral rosette, crowded on and tightly clasping a central stem. The fruit is a

[12] A.K. Maiti, et.al., "Application Of Natural Tenderizers In Meat- A Review" in http://www.arccjournals.com/pdf/Reviews/ar-29-3/ar-29-3-010.pdf, accessed on January, 12nd 2013

terminal cylindrical, compound structure at the apex of the stem and is formed by the fusion of the berry like fruitletstha develop from the flowers. At its apex, the fruit bears a compressed, leaf shoot called crown. The tipically yellow flesh is best eaten when sweet and moderately acid and may contain 10-18% sugar and 0,5-1,6% titratable acidity (Bartholomew et al.2003).[13]

The pineapple is a herbaceous perennial which grows to 1.0 to 1.5 meters (3.3 to 4.9 ft) tall, although sometimes it can be taller. In appearance, the plant itself has a short, stocky stem with tough, waxy leaves. When creating its fruit, it usually produces up to 200 flowers, although some large-fruited cultivars can exceed this. Once it flowers, the individual fruits of the flowers join together to create what is commonly referred to as a pineapple. After the first fruit is produced, side shoots (called 'suckers' by commercial growers) are produced in the leaf axils of the main stem. These may be removed for propagation, or left to produce additional fruits on the original plant.[4] Commercially, suckers that appear around the base are cultivated. It has 30 or more long, narrow, fleshy, trough-shaped leaves with sharp spines along the margins that are 30 to 100 centimeters (1.0 to 3.3 ft) long, surrounding a thick stem. In the first year of growth, the axis lengthens and thickens, bearing numerous leaves in close spirals. After 12 to 20 months, the stem grows into a spike-like inflorescence up to 15 cm (6 in) long with over 100 spirally arranged, trimerous flowers, each subtended by a bract. Flower colors vary, depending on variety, from lavender, through light purple to red.[14] The picture of pinepple *Ananas Comosus (L) Merr* is showed on Figure 2.1.

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[13] Majeed Mohammed, *Optimizing Posthharvest handling and Maintaining Quality of Fresh Pineapples (Ananas Cosmosus (L)*, (Tobago: Morton Publishing, 2004), p.1

[14] http://en.wikipedia.org/wiki/Pineapple , accessed on January, 11st 2013

The Scientific Classification of *Ananas Comosus (L) Merr* :

Kingdom	: *Plantae*
Devision	: *Angiosperms*
Class	: *Monocots*
Order	: *Poales*
Family	: *Bromeliaceae*
Subfamily	: *Bromeliaceae*
Genus	: *Ananas*
Species	: *A. Comosus*[15]

Fig. 2.1. Pineapple *(Ananas Cosmosus (L)*

Cultivated types of pineapple are called “clones”, because they are vegetatively propagated. There are many name clones, classed in 4-5 groups including “Cayenne”,”Spanish”, “Queen”, and “Pernambuco” which may represent botanical varieties. Commercial production is based mainly on clones in the “Cayenne” group, also known as “Smooth Cayenne”.[16] The morphology of Smooth Cayenne is appropriated on Figure 2.2.

[15] http://en.wikipedia.org/wiki/Pineapple , accessed on January, 11st 2013

[16] Majeed Mohammed, *Optimizing Posthharvest handling and Maintaining Quality of Fresh Pineapples (Ananas Cosmosus (L)*, p.1.

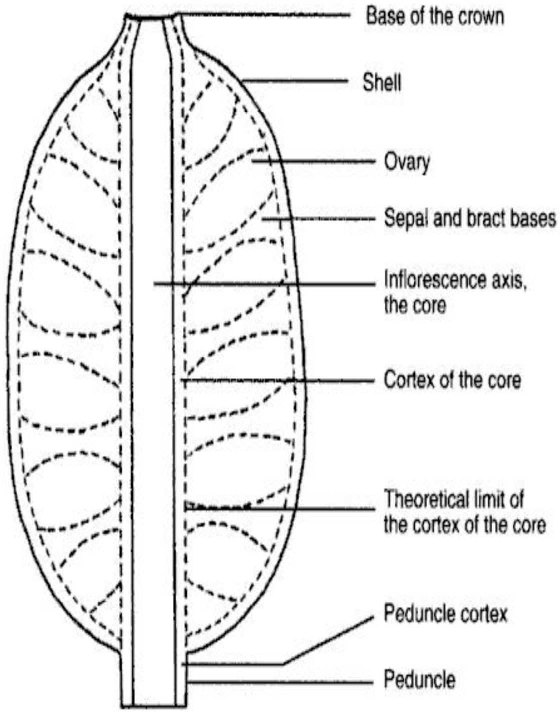

Fig.2.2 the morphology of *Smooth Cayenne*

Pineapple mainly contains water, carbohydrates, sugars, vitamins A, C and carotene, beta. It contains low amounts of protein, fat, ash and fibre. Pineapples contain antioxidants namely flavonoids, vitamin A and C. These antioxidants reduce the oxidative damage such as that caused by free radicals and chelating metals. It also has the enzyme complex protease (bromelain). Bromelain contains peroxidase, acid phosphate, several protease inhibitors and organically bound calcium. Chemical composition of the edible portion of pineapple (all varieties) (the data from USDA Nutrient Database) is showed on Table 2.1.[17]

[17] May Pitakere, http://foodscience.wikispaces.com/Pineapple, accessed on January, 14th 2013

Table 2.1. Composition of the edible portion of pineapple (all varieties) (the data from USDA Nutrient Database)

Nutrient	Units	Value Per 100 grams
Proximates		
Water	g	86.00
Protein	g	0.54
Total Lipid	g	0.12
Ash	g	0.22
Carbohydrate, by difference	g	13.12
Fiber, total dietary	g	1.4
Sugars, total	g	9.85
Sucrose	g	9.85
Glucose (dextrose)	g	5.99
Fructose	g	1.73
Vitamins		
Vitamin C, total ascorbic acid	mg	47.8
Vitamin A, IU	IU	58
Vitamin A, RAE	mcg_RAE	3
Other		
Carotene, beta	mcg	35

In the Eni Fajrin thesis entitled "*Application of the Bromelain Enzyme in the Making of Coconut (Cocos nucifera) Oil Enzymatically*", 2012, mentions the composition of bromelain enzyme in the plant of pineapple on Table 2.2.[18]

Table 2.2. composition of bromelain enzyme in the pineapple plant

[18] Eni Fajrin, "Application of the Bromelain Enzyme in the Making of Coconut (Cocos nucifera) Oil Enzymatically", thesis (Makassar: Agriculture faculty, Univesity of Hasanuddin Makassar, 2012), p. 7

No	Part of fruit	Percent
1	Ready fruit	0.060 – 0.080
2	Flesh of ready fruit	0.080 – 0.125
3	peel	0.050 – 0.075
4	stalk	0.040 – 0.060
5	Stem	0.100 – 0.600
6	Unripe fruit	0.040 – 0.060

In brief, pineapple (*Ananas Comosus (L) Merr*) is member of the diverse family Bromeliaceae. It indicates that pineapple contain protelytic enzyme or protease called Bromelain. Bromelain enzyme can be obtained from peel, stalk, stem, and flesh with different quantity.

3. Bromelain Enzyme

Bromelain is mixture of proteolytic enzymes extracted from the fruit and stem of pinepple plant, *Ananas Comosus*. Bromelain was initially named for any proteases obtained from various species of Bromeliaceae. Bromelain also contains small amounts of other physically active compounds, such as comosain, ananain, and other unidentified components.[19]

Bromelain is a proteolytic enzyme or protease. Such enzymes catalyze the breakdown of proteins into their amino acid building blocks through a hydrolysis reaction. **Hydrolysis** (L. *hydro* = water; Gk. *lysis* = loosening or breakdown) is the decomposition of large molecules into smaller units by combining them with water. In the case of protein digestion, the peptide bonds are broken with the insertion of the

[19] Melissa Pei Shian, "Pilot scale extraction of proteolytic enzyme bromelain from pineapple *(ananas comosus)*", in 2nd International Conference on Chemical and Bioprocess Engineering in conjunction with 19th Symposium of Malaysian Chemical Engineers, (malaysia: Departement of Bioprocess Engineering, fakulty of Chemichal and natural resources Engineeering, University of Technology Malaysia, 2005), p. 1-2

components of water, -H and -OH, at the cleaved (broken) ends of the chain.[20]

Bromelain is a protein which functions as an enzyme and belongs to a subclass of enzymes known as proteolytic enzymes (proteases). The function of proteases is to catalyze the hydrolysis of proteins to give amino acids. It is shown on figure 2.3.

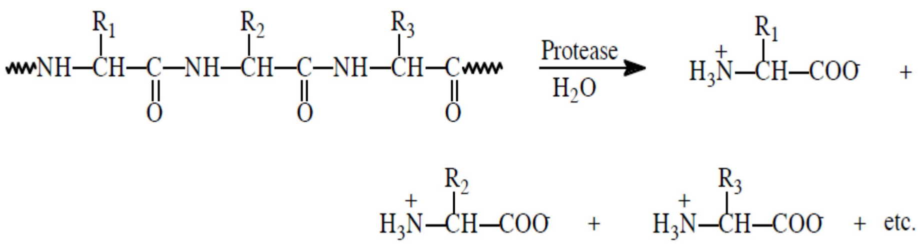

Fig. 2.3 reaction of proteolytic enzyme.

Proteolytic enzymes often catalyze self-degradation, but this usually occurs at a slower rate than the breakdown of other proteins.[21]

Bromelain is a crude, aqueous extract from the stems and immature fruits of pineapples (*Ananas comosus* Merr), mainly var. Cayenne from the family of bromeliaceae), constituting an unusually complex mixture of different thiol-endopeptidases and other not yet completely characterized components such as phosphatases, glucosidases, peroxidases, cellulases, glycoproteins and carbohydrates, among others.[22]

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[20] William V, et.al., "Using Bromelain in Pineapple Juice to InvestigateEnzyme Function", in http://www.organic-center.org/reportfiles/TasteReport.pdf, accessed on January, 12nd 2013

[21] Anonim, "Isolation and Caracterazitaton of Bromelain from Pineapple fruit", in http://faculty.swosu.edu/jason.johnson/pdf/4124lab3.pdf, accessed on January, 12nd 2013

[22] H. R. Maurer, "Bromelain: biochemistry, pharmacology and medical use", in http://www.volopharm.de/daten/Bromelain%20biochemistry,%20pharmacology%20and%20medical%20use.pdf, accessed on January, 12nd 2013

To sump up, Bromelain is crude, aqeous extract of pineapple fruit. Bromelain is one of kind from proteolytic enzyme which can breakdown proteins into their amino acid building blocks through a hydrolysis reaction.

4. Organoleptic Test

Organoleptic testing is generally use to verify the conformity of the seasoning to specification. These tests include flavor, taste, texture, aroma, and overal mouthfeel sensation when the product is consumed. The seasoning may be tasted as is, or in a liquid slurry form. A sample of the batch is also kept to compare to future batches to check for consistency. The standard will degrade over time, so it is necessary to obtain a fresh sample occasionally based on the customer's need.[23]

To support some tests that have been conducted, organoleptic test is needed in this research. Because organoleptic testing is generally used to verify the conformity of the seasoning to specification.

C. Hypothesis

The hypothesis of this research is waste of pineapple's peel (*Ananas Comosus (L) Merr*) as the source of bromelain enzyme can inrease meat (local beef) quality.

[23] Y.H.Hui, et.al., *Meat Science and Applications,* (New York: Marcel Dekker.Inc, 2005), p.385