KENYATTA UNIVERSITY, NAIROBI, KENYA

EFFECT OF PINEAPPLE (*Ananas comosus* L. Merrill) AND PAPAYA (*Carica papaya* L.) FRUIT EXTRACTS ON SERICIN REMOVAL FROM SILK MOTHS COCOONS IN KENYA

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university or any other award.

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DEDICATION

This thesis is dedicated to my late Dad Walter and my dear mum Priscilla who made me the person I am today and to my daughter June for making the sacrifice worthwhile.

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ABSTRACT

Sericulture (Silk farming) is the rearing of silk moths for the production of raw silk. Silk farming originated from China where it was secretly practiced for many years before spreading to other parts of the world. Silk moths are grouped into mulberry silk moths (domesticated) and wild silk moths. Silk moth cocoon has a double filament made of insoluble protein known as fibroin which is embedded in a water soluble protein called sericin. Prior to processing cocoons, are boiled in strong alkali so as to dissolve the sericin a process called degumming. Proteinases enzymes such as papain from papaya (*Carica papaya*) and bromelain from pineapple (*Ananas cosmosus*) characterised by their proteolytic activity have the potential to hydrolyse sericin, thus increasing its solubility in water. This study sought to investigate the effect of extracts from pineapple and papaya on the solubility of sericin protein from cocoons of three species of silk moths: *Gonometa postica*, *Argema mimosa*e and *Bombyx mori* at different temperatures. Twenty cocoons of each species were cut open, cleaned to remove the pupae remains and weighed. The cleaned cocoons were immersed in the degumming agents at various temperatures and five cocoons were taken out at 30 minutes intervals for 120 minutes. These cocoons were dried for two hours and then re-weighed. The degumming agents used were crude extracts from papaya and pineapple, commercial papain, commercial bromelain and their effects were compared with that of the conventionally used sodium bicarbonate and distilled water. The efficiency of the degumming processes was assessed using mean weight loss at different temperatures and was subjected to analysis of variance (ANOVA). Where significant difference existed, mean separation was carried out using the Student-Newman-Keuls test. In *G. postica* cocoons, papain gave the highest mean weight loss at a temperature of 60°C for all time intervals. The highest mean weight loss from *G.postica* was 0.5 g at 60 °C, 120 minutes in *G. postica* cocoons this was significantly different from the mean weight loss with other treatments. The least mean weight loss in *G. postica* was recorded in water and ripe papaya treatment which was significantly lower than the other treatments extract. *A.mimosae* cocoons showed highest weight loss with bromelain at 60°C, 90 minutes of but this was not significantly different with sodium bicarbonate. The least mean weight loss in *A mimosae* was recorded in papain and ripe papaya treatment 70°C, 120 minutes but this was not significantly lower than the other treatments extract. With *B. mori* cocoons, papain gave the highest mean weight loss of 0.12g at a temperature and time of 70° C, 60 minutes respectively, this was significantly different compared with the mean weight loss with all the other treatments. The results obtained from this study have shown that plant proteases can be utilised for effective degumming of cocoons. Commercial proteases were the best agent for degumming cocoons of *G. postica* and *B. mori* as it gave the highest mean sericin loss within the shortest duration. The results show that locally available fruit proteases can be used to remove sericin from silk moth cocoons. These offer farmers a cheaper local option while also shortening the time required for boiling hence saving fuel.

CHAPTER ONE

1.0 INTRODUCTION

1.1Background information

Sericulture (silk farming) is the rearing of silk moths for the production of raw silk. Silk farming originated from China. It was practiced secretly for many years before spreading to other parts of the world. Silk moths are grouped into mulberry silk moths (domesticated) and wild silk moths.

Many species belonging to the class Insecta spin cocoons in which they pupate. *Bombyx mori* (Lepidoptera: Bombycidae) is a classic example. This domesticated silk moths caterpillar spins its cocoon after feeding on mulberry leaves for nearly a month. Wild silk moths found in Kenya include *Gonometa postica* Walker (Lasiocampidae) found on acacia plant, and *Argema mimosae* (Boisduval), commonly known as African lunar moth among others (Kioko *et al*., 2000).

Silk is a product of specialised glands of some lepidopteran insect larvae and some arachnids and is used in the construction of cocoons. It is composed of two proteins known as fibroin and sericin (Romoser, 1973). Silk fibroins are the extracellular proteinaceous filaments produced by certain species with classes Insecta and Arachnida of the phylum Arthropoda. Sericin is a protein that serves as an adhesive that unite the fibroin for making of the silk moths cocoons. (Howitt, 1946., Lucas *et al*., 1958., Rundall, 1960)

The fibroin which is secreted from the posterior part of the silk gland is formed from amino acids present in the cells of the posterior part of the gland and from

the free amino acids present in the body fluid while sericin is secreted by the middle part of the gland. Removal of sericin from silk fibroin is accomplished by a process of degumming usually by one of the three methods: (1) extraction with water at high temperatures, (2) extraction with dilute aqueous alkali or soap solutions, or (3) removal by proteolytic enzymes (Raina, 2004).

A fundamental distinction between fibroin and sericin is the composition of amino acids that make up these two proteins. Characteristic features of fibroins in general are the high proportion of the smaller side group of the polymer amino acids such as glycine, alanine, and serine and their inability to dissolve in water. The amino acids allow a close-packing arrangement of the molecules which is a characteristic of the fibroins and is a general feature of extra cellular matrix proteins. Sericin, on the other hand, has over 30 % serine molecules (Komatsu, 1980) and is readily soluble in hot dilute alkali solutions (Lucas *et al*., 1960). Fibroin forms the core of the filament and is surrounded by sericin (Hiyabayashi *et al*.,1994).

Sericin is insoluble in cold water or in weak alkali. Acid, alkaline and enzymes can accelerate the hydrolysis of sericin. Approximately 4000 years ago in China it was discovered that by boiling a cocoon, the filaments used to construct the cocoon became loose and could be unwound in a single strand (Clausen, 1954). When several of these filaments are woven together from this thread, a soft lustrous yarn is the result. Presently, fibroin protein is used to make beautiful silk

cloth and it is also being actively researched on for use in cosmetics, food and biological material (Hiyabayashi *et al*.,1994.; Liu *et al*., 1998).

The use of enzymes in the silk industry is relatively unexplored and has generated a lot of interest and much research is being carried out internationally (Gulrajani *et al*. 2000). Proteinases, which are enzymes characterized by their proteolytic activity have the potential to effect partial solubility of the proteinaceous gum sericin which is involved in binding silk strands together in cocoon, an essential process in the silk cocoon cooking and reeling. As such, a pineapple fruit pulp extract having proteolytic activity due to the presence of cysteine proteinases can be used in the degumming of silkworm cocoons (Rowan and Buttle, 1994). Proteolytic enzymes like papain present in the latex of *Carica papaya* have also been extensively studied (Brocklehurst *et al*., 1981.; Thomas, 1994., Mellor, 1993).

Silk is composed of protein chains with the well-known basic amino acids. It is interesting to note that sericin and fibroin are composed of practically the same amino acids. This makes the enzymatic removal of sericin difficult without altering the fibroin. However, an opportunity presents itself due to the fact that the sericin covers the fibroin thus making it more accessible. Fibroin is also more crystalline than the sericin.

Degumming is the process employed to remove the silk gum (sericin) enveloping the two raw silk filaments (fibroin). Degumming is effected by careful boiling-off

in soap baths, which should only be slightly alkaline in order to avoid damaging the fibroin. Many investigators have carried out studies on degumming with soap for more than 200 years. In 1918, Kanegurachi cotton spinning Co. used an enzyme which was obtained from a vegetable oil filter cake or from an animal body tissue known as proteolytic enzyme for the degumming of raw silk. Shukla *et al*. (1992) degummed Tussah and Mulberry silk using enzymatic and alkaline methods. The results were calculated in terms of weight loss and tensile strength. Most of the mentioned degumming processes impose a markedly unnatural environment on the silk; therefore, one should consider the possibility that, changes could occur in the fibroin structure and its mechanical properties (Asakura and Kaplan, 1994).

As far as the environment is concerned, the utilization of chemicals by most of the mentioned methods introduces serious pollution to the receiving waters. Among these methods, only the enzymatic method has the ability to react with specific sites of the sericin. Consequently, through a controlled process one can avoid the shortcomings such as dyeing and tensile strength. (Gulrajani *et al.*,2000).

Enzyme degumming involves the proteolytic degradation of sericin, using the specific proteins with minimum effect on fibroin. Enzymes are selective and biodegradable, and there is no soap required in the enzymatic degumming process; therefore, uneven dyeing problems caused by metallic soap can be avoided. The affinity of silk to dyes, especially the reactive dyes, is significantly improved by the enzymatic treatment (Shenai and Saraf, 1993). The application of enzymes in

textile industries has recently been increased (Duran and Duran, 2000; Ramachandran and Karthik, 2004; Gupta *et al.,* 2000). Enzymes are eco-friendly products, operate under mild conditions and low temperature, and so consume less energy than other methods (Gulrajani *et al.*, 1990; Shukla *et al.*, 1992; Freddi *et al*., 1996 ; Gulrajani and Sen, 1998). Freddi *et al.*, (2003) used proteolytic enzymes (alkaline, neutral, and acidic proteases) for silk degumming and found that alkaline and neutral proteases effectively degummed crepe silk fabric and that the degumming kinetics depended on the enzyme dosage and treatment time.

Chopra and Gulrajani (1994) compared the effect of various degumming agents like alkali, acid and enzyme on degumming weight loss rate of degumming and changes in mechanical properties. They used China silk, Bangalore silk and Murshidabad silk to carry out experiments and found that the weight loss in all degumming processes was within the same range. Gulrajani and Gupta (1995) treated silk with protease and cellulase and reported that the treatment with cellulase improved wettability and also removed the impurities.

1.2 Justification of the study

Income generating initiatives are needed for the Kenya's rapidly growing population especially in the rural communities. Wild silk production which is eco-friendly and agro-based venture has a great potential for the environmental protection, employment generation, artisan's development and export earning. Besides the obvious immediate and positive effects, organic or natural farming has on the environment and quality of silk yarn, it will also greatly help a farmer to

become self-sufficient in requirements for agro – inputs by use of readily available material thus making the farmer self- reliant and reducing the input costs. Small scale hand spinning and weaving using very basic equipment and methods is still not a practical option due to the high cost of chemicals that are needed for the processing of cocoons including degumming of the silk yarn considering the economic status of most of the farmers. Hence, the need for adoption of the use of the locally available materials with minimum cost to enable the farmer to start and maintain sustainable nature based enterprise.

Sericulture is an enterprise that can give additional income to the rural poor and also enhance conservation of host plants and the wild silk moth species. In addition it can provide a new type of raw material for use in home industry and in addition, profitability of any enterprise is a key to its success. To this end, the evaluation of marketing strategies, product quality enhancement, and sustainability of production should be addressed (Raina, 2000). This study sought to investigate the effect of extracts from pineapple *(Ananas cosmosus*), papaya (*Carica papaya*) commercial bromelain and papain on the solubility of sericin protein at different temperatures and to determine the best agent for dissolution of sericin to facilitate reeling thus reducing the cost of processing of cocoons. Three species of the silk moths found in Kenya that included *Gonometa postica*, *Argema mimosa*e and *Bombyx mori* were used.

1.3 Research hypotheses

(a) There are no differences in the solubility of sericin from cocoons of *G. postica, A. mimosae* and *B. mori* at 60^0C and at 70^0C and at different duration when soaked in commercial bromelain and papain.

(b) There are no differences in the solubility of sericin from cocoons of *G. postica, A. mimosae* and *B. mori* at 60^0C and 70^0C and at different duration when soaked in crude raw, ripe papaya and pineapple extracts.

(c) There are no differences in solubility of sericin from cocoons of *G. postica, A. mimosae* and *B. mori* when soaked in extract of papaya and pineapple, bromelain and papain compared with the control (sodium bicarbonate and distilled water).

1.4 Objectives of the study

1.4.1 General objective

To determine the efficiency of enzymes found in crude pineapple and papaya fruit extracts, commercial bromelain and papain in the removal of sericin from silkworm cocoons.

1.4.2 Specific objectives

a) To determine sericin loss from cocoons of *Gonometa postica, Argema mimosae* and *Bombyx mori* when soaked in commercial papain and commercial bromelain at 60^0C and 70^0C for a duration of 120 minutes.

b) To determine the sericin loss from cocoons of *G. postica, A. mimosae* and *B. mori* when soaked in crude extract from pineapple and papaya at 60^0C and 70^0C for a duration of 120 minutes.

c) To identify the most effective degumming agent for cocoons of *G. postica*, *A. mimosae* and *B. mori* between the crude fruit extract and the commercial proteases and the optimum temperature.

1.4.3 Significance of the study

A shorter duration for the dissolution of sericin to enhance processing of the softened cocoon for spinning will reduces the cost of processing the cocoons and therefore can be used as an economical and practical procedure of degumming cocoons of silkmoths by local farmers in Kenya. The use of locally available materials which are also environmentally friendly should lead to effective utilisation of the silkmoths cocoons.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Economic importance of insects

Insects are notorious pests and vectors of diseases and their potential benefit to man and environment has often been overlooked. Amongst the many activities that might assist many farmers in Africa to escape their vicious cycle of poverty, is the production of silk from silk moths. The current dependence on primary production, largely in agriculture to sustain demands for food and income generation to improve the people's living standards is limited and has largely contributed to the dwindling of Africa's rich biodiversity (Oberprieler, 1995; Kioko *et al*., 1999, 2000; Raina and Kioko, 2000; Rahab, 2005; Selehe, 2005). It has also been reported that the immense commercialisation of agriculture has also had a very negative effect on the environment. The use of pesticides and chemicals has led to enormous levels of chemical build-up in our environment, especially in soil, water, air and in animals and even in our own bodies hence the need to look for environmental friendly alternatives such as the ones addressed in this study.

2.2 Sericulture

Sericulture, or silk farming, is the rearing of silkworms for the production of raw silk. The silk is a continuous-filament fiber consisting of fibroin protein, secreted from two salivary_glands in the head of each larva, and a gum called sericin, which cements the two filaments together. During the caterpillar phase in some lepidopteran larvae, the worm wraps itself in a liquid protein secreted by two large

silk glands in its head. This secreted protein hardens upon exposure to the air. The resulting silk fibroin of the cocoon is held together by gum-like protein known as sericin. Sericin is primarily amorphous and acts as a gum binder to maintain the structural integrity of the cocoon, so sericin is more water-soluble than fibroin (Karmakar 1999). This difference makes the gum easily removable from the filaments through various processes without considerable damage to the filaments.

Silk has always been the most cherished and sought-after of all the textile fibres. The processed silk fibre accounts for only 0.2 percent of the total world textile production while the raw silk accounts for 0.33 percent of natural fibre (Anonymous, 1999). The origins of sericulture and silk production are closely associated with the emergence of China as one of the great civilizations. It is believed that sericulture evolved gradually and by the middle of the third millennium BC, it was already being used by humanity. Because of its natural texture, strength and fitness, silk is one of the most ancient and preferred fibre in the world. The economic value of silk is higher than that of other natural fibres such as cotton and wool (Crotch, 1956)

The silk filament is a continuous thread of great strength measuring from 500-1 500 metres in length. Single filaments are too thin for utilization. For production purposes, several filaments are combined with a slight twist into one strand. This process is known as silk reeling. Not all the silk filament is usable for reeled silk. The left over silk cocoon include the brushed end, broken cocoons and floss from wild silk moth, this shorter staple silk may be used for spinning silk in a manner

of fabric like cotton and linen. Sericulture rightly fits into the social economic structure of rural areas and can serve as an effective tool for rural development (Das, 1993).

2.3 Processing of silkmoths cocoons

The various processes involved in the raw silk manufacture include removal of sericin, reeling and re-reeling. In reeling (removal of silk from the cocoon), the larva is killed in the cocoon by steam or hot air in the chrysalis stage before its metamorphosis. Removal of sericin is accomplished by a process of degumming which involves the boiling of cocoons in water under pressure at 115 ºC. However, for this reason this treatment gives a risk of fibroin being damaged when the time of treatment is prolonged. At the same time the process gives incomplete degumming. Sometimes soap or synthetic detergent must be added in order to improve the degumming effect (Gulrajani, 1992). Marseilles soap and olive oil soap are used at 98º C (Brag, 1929) but as a result of high temperature this process tends to attach both sericin and fibroin. In addition, this process requires soft water in order to avoid the formation of scum. Degumming with acid such as hydrochloric acid and citric acid has not received much attention in silk industry since alkaline solution is safer for fibroin than acids. Prior to reeling in *Bombyx mori*, cocoons are normally boiled in water to dissolve the soluble protein sericin which sticks the cocoon filaments together in the shell. The cocoons soften, swell and the filament is easily taken out and wound on a reel. The twisted silk fibre has 18-19% sericin gum. During degumming, the twisted silk hanks are placed in soapy water for 30 minutes. They are later immersed in boiling water

with 5% sodium bicarbonate for one hour. After degumming, the yarn is washed in clean water, and the fibre is soaked for 5 minute in 2% hydrogen peroxide solution prepared in boiling water. The yarn is then rinsed in clean water. Sustained heat processing softens the hardened sericin so that the filament can be unwound (Hisao, 1994).

Enzyme degumming has the following advantages (Gulrajani, 1992) over the conventional methods. It has lesser risk of over degumming than the alkaline soap degumming and the weight loss can be easily modified by adjusting the concentration of enzymes, the reaction time and the use of optimum pH and temperature. Enzyme treatment is environmentally friendly because the enzyme is readily biodegradable in nature. The process which is carried out at low temperature not only reduces energy costs but it also prevents fibre weakness and since no soap is used, there is no uneven degumming that may affect dyeing (Mokhtar *et al.,* 2006).

2.4 Silk fibre

Single silk filaments are too thin for utilization. Consequently for production purposes, several filaments are combined with a slight twist into one strand. This process is known as silk reeling or filature. Silk is a premium priced agricultural commodity, although its sheer volume is less than one percent of the market for natural textile fibres. The international demand for high quality silk has multiplied and appropriate cocoon-drying techniques and reeling operations are vital in supplying good quality silk. Although new technologies are applied among major

silk producing countries in Asia, many newcomers to the industry including non-traditional producers have not been transitioned to the methods that ensure high quality silk for export (Hisao, 1994). Spun silk is less expensive than reeled silk. Although spun silk has less strength and elasticity than reeled silk because of the shorter staple used, it possesses all the general characteristics of reeled silk. Tub silk fabric, for example, is made of spun silk, yet it gives good service when the quality of the fibre is good. Spun silk is used for shantung, pile fabrics, dress trimmings and linings, elastic webbing, sewing silk, summer weight silks, velvets, umbrella fabrics and insulation. Floss silk may be manufactured from any kind of cocoons, but principally it is processed from pierced, end-missing, and double cocoons (Hisao, 1994). Floss silk is beneficial as paddy against cold weather and as a basis for hand spun yarns. The procedure to create floss silk involves degumming, opening-up and finishing. The main purpose of degumming is to remove sericin from the cocoons and to reveal the lustre of the fibroin and to improve the appearance of the fibre (Saligram *et al*., 1993). When sericin is removed from the fibre, the fibroin must not be damaged at the same time. Raw silk of *Bombyx mori* is off- white to yellow in colour while the wild silk is uneven, brown and slightly less lustrous than those from cultivated silks.

2.5 Sericulture in Kenya

Silk production was first introduced in Kenya in 1904 but remained the business of a few individual farmers until 1973, when the Ministry of Agriculture (MOA) set up a Sericulture Research and Development Project with the aim of establishing commercial silk production. The major government support for the

development of silk industry was given in 1974, when the crop production division of MOA established sericulture Research Station in Thika. The Government of Japan, which supported the program supplied materials and sericulture experts. In order to accomplish this task, preliminary research to identify indigenous mulberry cultivars was carried out. The cultivars were imported from Japan and India in order to assess their performance in the Kenyan climatic condition (Waweru, 2000).The objectives of the Government of Kenya in support of sericulture are as follows:

a) A long term objective to increase the country foreign exchange earning through export of finished silk products and to generate additional and gainful employment in the rural areas.
b) To develop a comprehensive sericulture programme involving mulberry cultivating, silkworm rearing, drying and processing of cocoons and the various aspects of reeling.

Not all the silk filament is usable for reeled silk. The left over silk cocoon include the brushed end or broken cocoons; this shorter staple silk may be used for spinning silk in a manner of fabric like cotton and linen. The quality of spun silk is slightly inferior to reeled silk in that it is a bit weaker and it tends to become fuzzy. The waste material from the spun silk can be used for making waste silk or silk noil, the coarse material is commonly used for draperies and upholstery.

2.6 Rearing of domesticated silk moth (*Bombyx mori)*

Domesticated silk worm (mulberry silk worm) is cared for and fed in the house throughout its life in open trays. It is placed in open boxes as it change first into chrysalis and then the silk moth. There it builds a protective support of silk fibre within which it spins the cocoon (plate 3.1c).

According to Omura (1973), there are three categories of the mulberry silkworm *(Bombyx mori)* larvae i.e. univoltine, bivoltine and multivoltine. Univoltine and bivoltine belong to the temperate zone while polyvoltine belongs to the tropics. The main difference between the tropical and temperate races is that temperate races undergo obligatory egg diapauses before hatching while polyvoltine egg usually hatches in ten days after being laid. According to Jolly (1986) one hectare of mulberry can generate remunerative employment for 12 to 13 persons through out the year. The highly desirable properties of the *Bombyx mori* silk fibres are due to the unique structure of their fibroin protein and the exceptional ability to absorb dyes.

2.7 Wild silk farming

The *Bombyx mori* silkworm is not the only silkworm that spins a silk cocoon that can be used to produce silk fabric for silk clothing. There are many other species of wild silk caterpillars that produce silk cocoons used in the production of silk fabrics, sometimes called “wild silks” because the silk caterpillars are allowed to live in the wild and complete their life cycle in the wild. Most wild silkworms are multivoltine, which means that they produce cocoons several times during the

year (Hartland-Rowe, 1992). Invertebrate and particularly insects comprise that vast majority of life forms on earth (Holloway and Stork, 1991) and are a significant component of faunal diversity that cannot be ignored when dealing with biodiversity conservation. For example different species of the African moth produce high quality silk of commercial value (Chickwenhere, 1992, Hartland – Rowe, 1992, Peigler, 1993). Wild silk moths sometimes called non mulberry moths are lepidopteran insects whose larvae are able to produce silk. They are generally not reared in captivity instead; local people collect cocoons from wild population of the moths. In some cases, a certain amount of rearing is done often outdoors with little or no protection of the larvae. Wild silk farming requires minimal expense when compared with other agricultural endeavours. Wild silk farming offers a financial opportunity to farmers living in areas where species of wild silkmoths occur in the forests. In Mwingi, it has been shown that an acacia plant with a canopy of about 3 m can support 200 *Gonometa postica* silkworms (Kioko 1998).A farmer with 100 acacia trees can raise 16,000 live cocoons (allowing for 20% mortality). Currently a farmer receives two hundred shillings for a kg of live cocoons (about 200 cocoons) hence the 16,000 cocoons can make 80 kg achieving Ksh 16,000. There are two silkworm seasons in a year. If the 80 kg of cocoons are converted into silk, 30 kg can make up to 200 m of silk, which can fetch up to Ksh 190,000 at the rate of Ksh 950 per meter. If further value addition is undertaken by converting the cloth into shirts and tops, it can give 95 pieces worth Ksh 247,000 (at a rate of Ksh 2600 per item) (Kioko *et al*., 2000; Raina *et al*., 2004).

Harvesting cocoons from wild population has several advantages over establishing an artificial rearing facility. No host plant plantations have to be established and eggs and larvae do not have to be intensively cared for (Snyman, 1993). The present utilization of wild silk moths in the world hardly accounts for 5% of the rich potential and most of the production is far from that of Far East counties. The steady demand for silk in all silk consuming countries like the united state of America and Japan provide excellent opportunities for any country to venture into wild silk production.

Wild silk moth caterpillars spin a silk that is different in texture and color from the domesticated *Bombyx mori* and their strands are shorter because they come from cocoons that have been damaged by the wild silk moth's emergence from the cocoon. Wild silk caterpillars secrete silk fibres that do not to accept dyes as well.

Exquisitely, patterned wild silk fabrics are hand loomed in Thailand from the Saturniidae silk worm, and throughout India from a variety of wild silk caterpillars. One region of India that is especially noted for its exceptional and unique silks is the far North Eastern state of Assam, which borders Bangladesh. Assam produces three different types of silk that are collectively known as Assam silk but which vary greatly in appearance (Thangavelu *et al*., 1988).

Muga silk from the semi-domesticated silkworm (*Antherea assamensis*) is renowned for its glossy fine texture, durability and natural golden amber glow. Reputed to be the second most costly fabric after Pashmina, Muga silk looks like spun gold. The golden hue increases with time and washing. Muga silk is

naturally stain-resistant and is never bleached or dyed. Like cotton and silk, fashion is a blending of compromises (Thangavelu *et al*., 1988).

Muga silk is spun from the cocoons of semi-domesticated silkworms because the *Antherea assamensis* silk caterpillars are not destroyed in the cocoon but are allowed to emerge as moths and live a full lifecycle (Jolly *et al*., 1979). Eri silk from the domesticated silkworm (*Philosamia ricini*) is a fine silk that is almost as white in color as the *Bombyx mori* silks but because the Eri silk fibers are more uneven and the cocoons are damaged when the moth emerges, it is spun rather than reeled. Eri silk has the look of wool mixed with cotton but has the feel and softness of silk. Muga and Eri silks are from silkworms that are only found in India.

Silk cocoon from *Gonometa postica* Walker (Lasiocampidae) has been reported to occur on the African savannah where larvae feed on the mopane tree (Hartland-Rowe, 1992). Cocoons are collected in the field and give silk of a soft texture and beige colour (Peigler, 1993). In Botswana, a study by Hartland-Rowe (1992) indicated that collecting and processing of this silk can offer a viable source of employment and income to people living in the village. In Zimbabwe, Chikwenhere (1992) reported that from 1986 to 1987, there were 430 tons of wild cocoons which were collected by rural families and this became a source of employment in the rural areas. It is recorded that indeed there is a factory of this silk in operation in Madagascar (Peigler, 1993). On international level, 10% of the yearly silk tonnage of wild silk in the world is produced by insects which are not domesticated (Schenk, 1981). The present exploitation hardly accounts for 5% of

the rich potential (Jolly, 1986) and most of the production is in the Far East countries. The steadily growing demand for silk in all the silk consuming countries indicates excellent opportunities for any country to increase her silk production (Kumar, 1995). In Africa, silk moths species, *Gonometa rufobrunnea* Aurivillius *and G. postica* Walker were discovered in the wild in Botswana in 1985 and attempts have been made to utilise their cocoons for production of silk (Hartland-Rowe, 1992). There is no major wild silk industries established in Kenya but there are training centres for farmers which have been established in Mwingi in eastern province, Kakamega forest and Arabuko Sokoke in Malindi (Kioko *et al.,* 2000).

2.7.1 *Argema mimosae*

Argema mimosae (Boisduval) commonly known as the African lunar moth or moon moth is a saturniid. It was described in 1847 as *Saturnia mimosae* from specimen's collected by French explorer Adulphine Delegorge in Natal, South Africa and larvae spin silvery cocoons. The cocoons have irregular small holes and a prominent emergent valve at the top end and are attached to the side on to the twig. Cocoons of the first generation of the moth are found in the field from May to October while the second generation is from December to March (Kioko *et al*., 2000).

2.7.2 *Gonometa postica*

In Kenya, *Gonometa postica* walker (Lasiocampidae) occurs in various parts and cocoons have been recorded in Kamaguti in Uasin Gishu District, Nguni in the

Mwingi District, and in Wote and Sultan Hamud in the Makueni District (Kioko *et al.*, 2000; Ngoka *et al*., 2008). The cocoons are found on twigs and are very hard; presumably because they contain a lot of sericin (Kioko *et al.*, 2000). The collecting and processing of the cocoon offers a source of employment and income to persons in the villages. The cocoons are used to make spun silk although they have other uses such as making of ankle rattles (Kioko *et al.*, 2000).

2.8 Cultivation and use of Papaya (*Carica papaya L)*

Papaya (*Carica papaya L*) is placed in the family Caricaceae it is a native of Central America but has been carried through out the tropics were it is extensively cultivated. Average life span of a papaya is about 25 years in the wild. Fresh fruits are harvested just as yellowing commences. When harvesting the fruits they are twisted gently not to bruise the fruit surface. Since papaya is injured by chilling, fruits are kept at about seven degrees centigrade with relative humidity of 85-90% and may be kept for 7-21 days under these conditions. Papain is harvested by tapping the unripe fruits. Latex drips into a suitable container and is sun dried or oven dried at 55 to 66 degree centigrade. The same fruits are tapped in different cuts at weekly intervals. Tapped fruits are ultimately edible, so that both fruit and latex are harvested. Trees produce about 50% of their papain yield in the first year, 30% in the second year and 20% in the third year. A tree may bear 30-150 fruits per year. Sri Lanka, Uganda and Tanzania are the principal producers of papain. United States is the principal importer of papain using it mainly as meat tenderiser, in beer treatment, chewing gum, and textile and tanning industry.

Following extraction of papain and its relative from green fruits, they can be used to produce pectin (Murthy and Natarajan, 1992).

In the tropics papaya is used for many purposes and where grown it is a staple food favoured as breakfast fruit, and as an ingredient in jelly, it is preserved or cooked in various ways and its juice makes a popular beverage. Present in the latex is a proteolytic enzyme known as papain. This enzyme has many industrial uses and has attracted high research interest. Among major applications of papain is its use in food industry (Neidlema, 1991) in beer clarification (Caygrill, 1979), meat tenderizing and preparation of protein hydrolysates (Dupaigne, 1973). Papain is also used in the degumming of natural silk, to clean silks and wools before dyeing and to remove hair from hides during tanning (Duke, 1984). It is also used in the manufacture of rubber from *Hevea* (Morton, 1977). Commercially, it is used in some dentifrices, shampoos, and face lifting preparation. It is also used to extract oil from tuna liver (Duke, 1984).

2.9 Cultivation and use of Pineapple (*Ananas comosus* L. Merrill)

Pineapple (*Ananas comosus* L. Merrill) is placed in the order Bromeliales and the family Bromeliaceae. Pineapple is cultivated mainly for its fruit that is consumed fresh or as canned fruit and juice. Pineapple is the only source of Bromelain, a proteolytic enzyme or protease. This enzyme will catalyse the breakdown of protein into amino acid building block through hydrolysis. Bromelain is used in pharmaceutical industries and as a meat tenderising agent. The fruit of pineapple *Ananas comosus* (L) Mer is a rich source of a mixture of cystein proteinases, the

most abundant among them being bromelain which hydrolytically cleaves the internal peptide bonds in protein with relative broad specificity (Rowan and Battle, 1994). The pineapple proteinases find uses in various industrial and medical application including brewing, meat tenderisation, and prevention of diarrhoea, digestive aid and treatment of oedema (Takagi *et al*., 1992; Tanabe *et al.*, 1996; Chandler and Mynott, 1998; Kelly, 1996; Maurer, 2001).

Stems and leaves of pineapple are also a source of fibre that is white, creamy and lustrous as silk. Pineapple fibre has been processed into paper with remarkable qualities of thinness, smoothness and pliability (Collins, 1960.; Montinola, 1991). Parts of the plant are used for silage and hay for cattle feed. Processing waste in the form of shell, core materials and centrifuged solids from juice production are also used as animal feed. Alcoholic beverages can also be made from the juice. Lishram *et al.* (2003) found that proteolytic activity exhibited by pineapple extract was enhanced by the addition of sodium carbonate during the cooking of tasar cocoons.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study site

The experiment was carried out at the International Centre of Insect Physiology and Ecology (*ICIPE*), Nairobi, Kenya. The campus is about 16 kilometres from the city centre, longitude 36 °, 48 °E and latitude 1 °, 16 ° south.

3.2 Preparation of pineapple extract

Pineapple (*Ananas comosus* (L) Merr.cv. Queen) fruits were obtained from the local market. The pulp was prepared from the fruits by first detaching the crown and stem parts and then slicing off the skin part. The fruit was then diced into small pieces and blended. The blended pineapple pulp (50g) was homogenized separately with distilled water (50ml). The homogenate was strained through a sieve and then each diluted by adding an equal amount of distilled water.

3.3 Preparation of papaya extract

Fresh, ripe and raw fruits were obtained from the local market. The papaya fruits were cut and the pulp scooped out separately from each fruit and then blended. The pulp (50g) was blended and homogenised separately with distilled water (50ml). The resulting homogenate was strained through a sieve and then diluted with equal amount (100ml) of distilled water.

3.4 Commercial Bromelain and Papain

Bromelain and papain were obtained from Sigma-Aldrich Inc. New Jersey U.S.A. and all the chemicals were laboratory grade. An enzyme concentration of 1g per litre was used during the treatment.

3.5 Silk cocoons

Cocoons of *Bombyx mori, Argema mimosae* and *Gonometa postica* were used in the study. *G. postica* and *A. mimosae* cocoons were obtained from Arabuko Sokoke forest while *B. mori* cocoons were obtained from *icipe* mulberry silkworm rearing laboratory. Samples of 20 cocoons of each species were used for each treatment with five replications for each treatment (plates 3.1a, 3.1b).

Plate 3.1a *Argema mimosae* cocoons

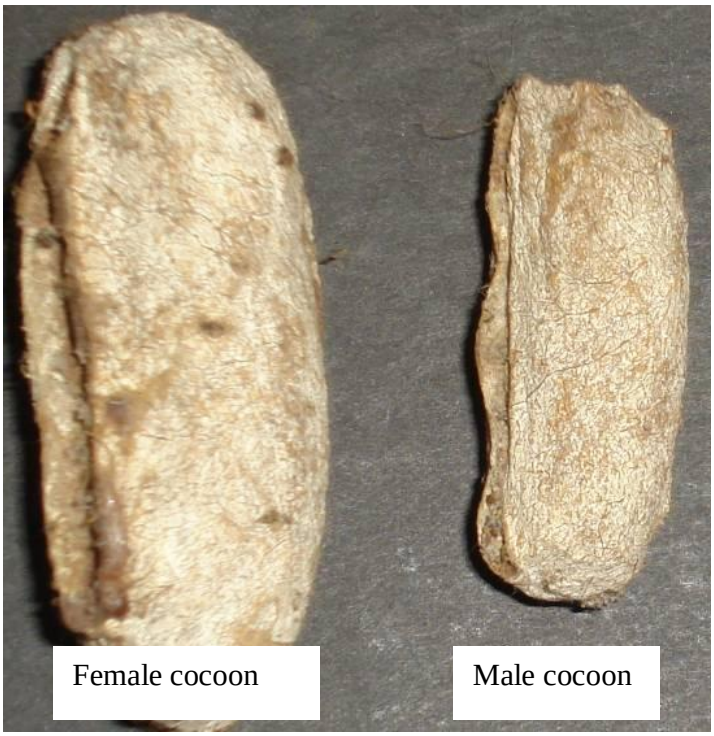

Plate 3.1b Female and Male *Gonometa postica* cocoons

Plate 3.1c *Bombyx mori* cocoons

3.6 Effect of temperature on weight loss of cocoons

The cocoon were cut and opened individually and turned inside out to remove pupae and other waste before weighing to determine the initial weight of the untreated cocoon. Twenty cocoons of each species in bunches of five were soaked in degumming agent in a water bath at 60^0C for 120 minutes. Samples of five cocoons were removed every 30 minutes and rinsed in water. The samples were oven dried at 95^0C till a constant weight was achieved and the procedure replicated five times. The weight loss was calculated (initial wt – final wt) and reported as the mean weight loss in grams. This procedure was repeated using pineapple and papaya extracts from ripe and raw fruits, papain, and bromelain. This procedure was then repeated at 70^0C and for the same duration.

3.6.1Determination of sericin loss

After the removal of pupae contents from the cocoons they were weighed and then subjected to each treatment per specified period of time, the samples were dried in an oven at 95^0 C to reach a constant weight and the weight loss determined as the difference in the weights of the cocoons before and after the treatment. The weight loss was taken to represent the amount of sericin lost in grams. This procedure was replicated five times.

3.6.2 Data Analysis

The effect of various treatment extracts and commercial enzymes were determined by calculating the mean weight loss of sericin at different temperatures before being subjected to analysis of variance using computer programs (SAS, 2000) and where significant differences existed, mean separation was carried out using the Student-Newman-Keuls test.

CHAPTER FOUR

4.0 RESULTS

4.1 Weight loss of *Gonometa postica* cocoons at 60 ºC and 70 ºC for 30 minutes

Bromelain and papain recorded the highest mean weight loss at 60 °C for 30 minutes followed by sodium bicarbonate, pineapple and water which showed no significant difference. These were followed by raw papaya which recorded significantly higher mean weight loss than the ripe papaya extract at 60 °C and 70 °C for 30 minutes. Increase in temperature to 70 °C had on effect on the mean weight loss in pineapple, sodium bicarbonate, bromelain and ripe papaya. While mean weight loss at 70 °C in water, raw papaya and papain were significantly lower. (p=0.05 (df=6,28 f=20.03 p =0.0001) (Table 4a).

Table 4.1a Mean weight loss (± SE) of *G. postica* cocoons at 60 ºC and 70 ºC for 30 minutes

Treatment	60^{0}C, 30 min	70^{0}C, 30 min
Water	0.18 ± 0.01^{aB}	0.14 ± 0.01^{bBC}
Pineapple	0.19 ± 0.01^{aB}	0.16 ± 0.03^{aB}
$NaHco_3$	0.20 ± 0.01^{aB}	0.16 ± 0.03^{aB}
Bromelain	0.28 ± 0.03^{aA}	0.31 ± 0.03^{aA}
Ripe papaya	0.07 ± 0.02^{aD}	0.05 ± 0.01^{aC}
Raw papaya	0.12 ± 0.01^{aC}	0.08 ± 0.02^{bBC}
Papain	0.25 ± 0.01^{aA}	0.13 ± 0.02^{bBC}

Means within a column followed by different upper case and each pair within the row followed by different lower case letter(s) are significantly different (p= 0.05 SNK)

Bromelain recorded significantly higher mean weight loss at 70 ºC for 30 minutes of 0.31±0.03g. This was followed by mean weight losses recorded from water, pineapple, raw papaya and papain. The mean weight loss recorded at 70 ºC, 30 minutes in ripe papaya treatment was significantly lower than mean weight loss from other treatments (p=0.05) (Table 4.1a).

4.1.1 Weight loss of *Gonometa postica* cocoons at 60 ºC and 70 ºC for 60 minutes.

Papain recorded the highest weight loss at 60 ºC, 60 minutes of 0.42g that was significantly different from the other treatments. It was followed by Bromelain and sodium bicarbonate treatments then raw papaya and pineapple extract treatments. The least mean weight loss was recorded in water and ripe papaya treatment which was significantly lower than the other treatments extract (p=0.05) (Df=6,28 f=21.21 p=0.0001) (Table 4b). A higher temperature of 70º C had no effect on mean weight loss from the treatments except with raw papaya were it was significantly lower. However, at 70º C, 60 minutes, bromelain and papain treatments recorded the highest weight however this was not significantly different from weight loss from the control (sodium bicarbonate). Mean weight loss from the other treatment were also not significantly different from the controls (sodium bicarbonate) (p=0.05) (Df=6, 28 f=21.21 p=0.0001) (Table 4b).

Table 4.1b Mean weight loss (± SE) of *G. postica* cocoons at 60 ºC and 70 ºC for 60 minutes

Treatment	60^{0}C, 60 min	70^{0}C, 60 min
Water	0.19 ± 0.02^{aC}	0.20 ± 0.02^{aB}
Pineapple	0.13 ± 0.03^{aCD}	0.15 ± 0.02^{aB}
$NaHco_3$	0.22 ± 0.02^{aBC}	0.27 ± 0.03^{aAB}
Bromelain	0.29 ± 0.03^{bB}	0.42 ± 0.05^{aA}
Ripe papaya	0.08 ± 0.02^{aD}	0.11 ± 0.01^{aB}
Raw papaya	0.17 ± 0.02^{aC}	0.12 ± 0.01^{bB}
Papain	0.42 ± 0.03^{aA}	0.36 ± 0.10^{aA}

Means within a column followed by different upper case and each pair within the row followed by different lower case letter(s) are significantly different (p= 0.05 SNK)

4.1.2 Weight loss of *Gonometa postica* cocoons at 60 ºC and 70 ºC for 90 minutes.

Mean weight loss of *G. postica* cocoons for all the treatments at 60º C and 90 minutes was not significantly different (df= 6,27 f=1.43,p=0.239) (Table 4.1c). Increase in temperature to 70º C gave no significant difference in mean weight loss observed compared to that at 60º C for all the treatments. However, at 70ºC and 90 minutes the mean weight loss from the cocoons with papain and sodium carbonate was significantly higher than that observed in water, pineapple, raw and ripe pawpaw extracts but not significantly different from the mean weight loss in bromelain (p=0.05) (Table 4.1c). Mean weight loss with ripe papaya was the least but this was not significantly different from mean weight loss from water, raw papaya and pineapple extract.

Table 4.1c Mean weight loss (± SE) of *G. postica* cocoons at 60 ºC and 70 ºC for 90 minutes.

Treatment	60^0C, 90 min	70^0C, 90 min
Water	0.23 ± 0.02^{aA}	0.17 ± 0.02^{aBC}
Pineapple	0.26 ± 0.02^{aA}	0.15 ± 0.02^{bBC}
$NaHco_3$	0.28 ± 0.01^{aA}	0.32 ± 0.05^{aA}
Bromelain	0.36 ± 0.06^{aA}	0.25 ± 0.05^{aAB}
Ripe papaya	0.09 ± 0.02^{aA}	0.10 ± 0.02^{aC}
Raw papaya	0.41 ± 0.21^{aA}	0.18 ± 0.04^{aBC}
Papain	0.31 ± 0.04^{aA}	0.34 ± 0.03^{aA}

Means within a column followed by different upper case and each pair within the row followed by different lower case letter(s) are significantly different (p=0.05 SNK)

4.1.3 Weight loss of *Gonometa postica* cocoons at 60 ºC and 70 ºC for 120 minutes.

At 120 minutes, 60 ºC the highest mean weight loss in the *G.postica* cocoons of 0.50 ± 0.04g was recorded with papain and this was significantly different from all other treatments (Table 4.1d). This was followed by mean weight loss from bromelain, raw papaya extract and pineapple extract but this was not significantly different that from water and sodium bicarbonate treatments. Ripe papaya extract had the lowest mean weight loss that was significantly different from all the other treatments. Increase of temperature to 70 ºC had no effect on the mean weight loss

in water, pineapple and ripe papaya extract, sodium bicarbonate and Bromelain but produced lower mean weight loss in raw papaya extract and papain.

At 120 minutes, 70º C bromelain papain and pineapple extract give the highest mean weight loss. However this was not significantly different from the mean weight loss from the other treatments or sodium bicarbonate (Df= 6,28, f=5.20, p=0.0011) (Appendix ih), (Table 41d).

Table 4.1 d Mean weight loss (± SE) of *G. postica* cocoons at 60 ºC and 70 ºC for 120 minutes.

Treatment	60^{0}C, 120 min	70^{0}C, 120 min
Water	0.21 ± 0.03^{aBC}	0.15 ± 0.03^{aB}
Pineapple	0.24 ± 0.03^{aBC}	0.25 ± 0.03^{aAB}
$NaHco_3$	0.31 ± 0.05^{aB}	0.42 ± 0.08^{aA}
Bromelain	0.25 ± 0.02^{aBC}	0.35 ± 0.08^{aAB}
Ripe papaya	0.13 ± 0.01^{aC}	0.16 ± 0.02^{aB}
Raw papaya	0.25 ± 0.01^{aBC}	0.15 ± 0.02^{bB}
Papain	0.50 ± 0.04^{aA}	0.33 ± 0.03^{bAB}

Means within a column followed by different upper case and each pair within the row followed by different lower case letter(s) are significantly different (p=0.05 SNK)

4.2 Weight loss of *Argema mimosae* cocoons at 60 ^{0}C and 70 ^{0}C for 30 minutes.

Mean weight losses of *Argema mimosae* cocoons at 30 minutes, 60º C and 70 º C were not significantly different (Table 4.2a). The highest mean weight loss of 0.10g occurred in sodium bicarbonate at 30 minutes 70 ºC.

Table 4.2a Mean weight loss (± SE) of *A. mimosae* cocoons at 60 ºC and 70 ºC for 30 minutes.

Treatment	60 ^{0}C ,30 min	70 ^{0}C, 30 min
Water	0.07 ± 0.004^{aA}	0.07 ± 0.005^{aA}
Pineapple	0.06 ± 0.009^{aA}	0.06 ± 0.006^{aA}
$NaHco_3$	0.06 ± 0.012^{aA}	0.10 ± 0.013^{aA}
Bromelain	0.07 ± 0.007^{aA}	0.07 ± 0.009^{aA}
Ripe papaya	0.05 ± 0.009^{bA}	0.08 ± 0.002^{aA}
Raw papaya	0.06 ± 0.009^{aA}	0.09 ± 0.007^{aA}
Papain	0.08 ± 0.009^{aA}	0.08 ± 0.007^{aA}

Means within a column followed by different upper case and each pair within the row followed by different lower case letter(s) are significantly different (p=0.05 SNK)

4.2.1 Weight loss of *Argema mimosae* cocoons at 60 ^{0}C and 70 ^{0}C for 60 minutes.

At 60 ºC, 60 minutes the highest mean weight loss from *Argema mimosae* cocoons was recorded was significantly different for all treatments (Table4.2b).

Increase of temperature to 70º C had no effect on the mean weight loss for all the treatments.

Table 4.2b Mean weight loss (± SE) of *A. mimosae* cocoons at 60 ºC and 70 ºC for 60 minutes.

Treatment	60 ^{0}C, 60 min	70 ^{0}C , 60 min
Water	0.07 ± 0.005^{aA}	0.09 ± 0.010^{aA}
Pineapple	0.07 ± 0.009^{aA}	0.08 ± 0.009^{aA}
$NaHco_3$	0.05 ± 0.006^{aA}	0.08 ± 0.009^{aA}
Bromelain	0.08 ± 0.007^{aA}	0.06 ± 0.007^{aA}
Ripe papaya	0.06 ± 0.033^{aA}	0.11 ± 0.004^{aA}
Raw papaya	0.07 ± 0.011^{aA}	0.07 ± 0.032^{aA}
Papain	0.09 ± 0.011^{aA}	0.13 ± 0.070^{aA}

Means within a column followed by different upper case and each pair within the row followed by different lower case letter(s) are significantly different (p=0.05 SNK)

4.2.2 Weight loss of *Argema mimosae* cocoons at 60 ^{0}C and 70 ^{0}C for 90 minutes.

The highest mean weight loss was recorded in bromelain at 90 minutes, 60 ºC of 0.12 ± 0.02g this was significantly different from mean weight loss from the other treatments except sodium bicarbonate.(table 4.2c) (Df= 6,28 f= 6.549= 0.002) (Appendix iie). Ripe papaya extract had the lowest mean weight loss that was significantly different from all the other treatments. Increase in temperature to

70ºC had no significant effect on the mean weight loss from the treatments except ripe papaya treatment were it was significantly higher at a higher temperature (Table 4.2c). No significant mean weight loss was observed in cocoons at 70º C, 90 minutes (Df= 6, 28 f= 0.50 p= 0.8012) (Table 4.2b).

Table 4.2c Mean weight loss (± SE) of *A. mimosae* cocoons at 60 ºC and 70 ºC for 90 minutes.

Treatment	$60\ ^{0}$C, 90 min	$70\ ^{0}$C, 90 min
Water	0.08 ± 0.003^{aBC}	0.08 ± 0.012^{aA}
Pineapple	0.06 ± 0.010^{aBC}	0.07 ± 0.010^{aA}
$NaHco_3$	0.09 ± 0.005^{aAB}	0.08 ± 0.015^{aA}
Bromelain	0.12 ± 0.020^{aA}	0.09 ± 0.010^{aA}
Ripe papaya	0.05 ± 0.006^{bC}	0.07 ± 0.007^{aA}
Raw papaya	0.06 ± 0.004^{aBC}	0.07 ± 0.006^{aA}
Papain	0.07 ± 0.010^{aBC}	0.07 ± 0.007^{aA}

Means within a column followed by different upper case and each pair within the row followed by different lower case letter(s) are significantly different (p=0.05 SNK)

4.2.3 Weight loss of *Argema mimosae* cocoons at 60 ^{0}C and 70 ^{0}C for 120 minutes.

At 60º C, 120 minutes mean weight loss in the cocoons did not differ significantly for all the treatment. (Table 4.2d). Increase of temperature to 70º C had no effect

on the mean weight loss from the cocoon (Table 4.2d). No significant mean weight loss was observed in cocoons at 70 ºC, 120 minutes.

Table 4.2d Mean weight loss (± SE) of *A. mimosae* cocoons at 60 ºC and 70 ºC for 120 minutes.

Treatment	60^0C, 120 min	70^0C, 120 min
Water	0.09 ± 0.010^{aA}	0.09 ± 0.026^{aA}
Pineapple	0.06 ± 0.014^{aA}	0.04 ± 0.007^{aA}
$NaHco_3$	0.07 ± 0.006^{aA}	0.05 ± 0.007^{aA}
Bromelain	0.08 ± 0.010^{aA}	0.10 ± 0.008^{aA}
Ripe papaya	0.07 ± 0.004^{aA}	0.03 ± 0.005^{aA}
Raw papaya	0.07 ± 0.007^{aA}	0.07 ± 0.005^{aA}
Papain	1.94 ± 1.860^{aA}	0.03 ± 0.005^{aA}

Means within a column followed by different upper case and each pair within the row followed by different lower case letter(s) are significantly different (p=0.05 SNK)

4.3 Weight loss of *Bombyx mori* cocoons at 60 ºC and 70 ºC for 30 minutes.

The highest significantly different mean weight loss from *Bombyx mori* cocoons at 30 minutes, 60^0C was recorded from papain followed by bromelain, this was significantly different from the control at 30 minutes, $60\ ^0$C (p=0.05) (Table 4.3). The lowest mean weight loss in the cocoons was recorded in the ripe papaya treatment but this was not different from weight loss from raw papaya and pineapple extract, water and sodium bicarbonate. Increase in temperature to 70 ºC

had no effect in papain, bromelain, pineapple and ripe papaya extract and water but gave significantly higher result in raw papaya extract and sodium bicarbonate treatments. At 70 ºC, 30 minutes papain followed by bromelain gave a significantly higher mean weight loss while ripe papaya gave the lowest mean weight loss although this was not significantly different from other treatments except papain and bromelain.

Table 4.3a Mean weight loss (± SE) of *B. mori* cocoons at 60 ºC and 70 ºC for 30 minutes.

Treatment	60^0C, 30 min	70^0C, 30 min
Water	0.02 ± 0.002^{aC}	0.03 ± 0.004^{aBC}
Pineapple	0.02 ± 0.003^{aC}	0.03 ± 0.01^{aBC}
$NaHco_3$	0.02 ± 0.002^{bC}	0.04 ± 0.004^{aBC}
Bromelain	0.04 ± 0.002^{aB}	0.04 ± 0.009^{aB}
Ripe papaya	0.01 ± 0.003^{aC}	0.02 ± 0.002^{aC}
Raw papaya	0.02 ± 0.003^{bC}	0.03 ± 0.01^{aBC}
Papain	0.09 ± 0.006^{aA}	0.09 ± 0.007^{aA}

Means within a column followed by different upper case and each pair within the row followed by different lower case letter(s) are significantly different (p=0.05 SNK)

4.3.1 Weight loss of *Bombyx mori* cocoons at 60^0C and 70ºC for 60 minutes.

The highest significantly different mean weight loss from *Bombyx mori* cocoons at 60^0C, 60 minutes was with papain and this was followed by bromelain, raw

papaya, sodium bicarbonate, pineapple and water. The lowest mean weight loss from the cocoons was recorded in the ripe papaya but this was significantly different from papain and bromelain (Table 4.3b). No significant difference was observed in the weight loss at 60º C and 70 ºC in 60 minutes for all the treatments other than in the ripe papaya treatments which had higher mean weight loss at higher temperature (Table 4.3b). At 70 ºC papain gave a significantly higher mean weight loss followed by sodium bicarbonate. Water gave a significantly lower mean weight loss than all the other treatments.

Table 4.3b Mean weight loss (± SE) of *B. mori* cocoons at 60 ºC and 70 ºC for 60 minutes.

Treatment	$60\ ^{0}$C, 60 min	$70\ ^{0}$C, 60 min
Water	0.02 ± 0.001^{aBC}	0.01 ± 0.007^{aD}
Pineapple	0.02 ± 0.004^{aBC}	0.03 ± 0.002^{aC}
$NaHco_3$	0.02 ± 0.001^{aBC}	0.05 ± 0.003^{aB}
Bromelain	0.04 ± 0.003^{aB}	0.03 ± 0.003^{aC}
Ripe papaya	0.01 ± 0.001^{bC}	0.03 ± 0.003^{aC}
Raw papaya	0.02 ± 0.002^{aBC}	0.03 ± 0.002^{aC}
Papain	0.10 ± 0.009^{aA}	0.12 ± 0.007^{aA}

Means within a column followed by different upper case and each pair within the row followed by different lower case letter(s) are significantly different (p=0.05 SNK)

4.3.2 Weight loss of *Bombyx mori* cocoons at 60 °C and 70 ºC for 90 minutes.

The highest mean weight loss in *B. mori* was with papain and this was significantly different from other treatments. At 60 ºC the lowest mean weight loss was from ripe papaya but this was not significantly from sodium bicarbonate, raw papaya, pineapple extract and water. A higher temperature had no effect on the mean weight loss for all the treatment except with sodium bicarbonate which gave a higher mean weight loss at 70 ºC. The mean weight loss of *B. mori* cocoons at 70 ºC, 90 minutes, was not significantly different in all the treatments (Table 4.3c).

Table 4.3c Mean weight loss (± SE) of *B. mori* cocoons at 60 ºC and 70 ºC for 90 minutes.

Treatment	$60\ ^{0}$C, 90 min	$70\ ^{0}$C, 90 min
Water	0.02 ± 0.003^{aBC}	0.02 ± 0.003^{aA}
Pineapple	0.02 ± 0.009^{aBC}	0.03 ± 0.003^{aA}
$NaHco_3$	0.02 ± 0.002^{bBC}	0.06 ± 0.01^{aA}
Bromelain	0.04 ± 0.003^{aB}	0.04 ± 0.002^{aA}
Ripe papaya	0.01 ± 0.001^{aC}	0.03 ± 0.003^{aA}
Raw papaya	0.02 ± 0.001^{aBC}	0.05 ± 0.02^{aA}
Papain	0.08 ± 0.007^{aA}	0.09 ± 0.04^{aA}

Means within a column followed by different upper case and each pair within the row followed by different lower case letter(s) are significantly different (p=0.05 SNK)

4.3.3 Weight loss of *Bombyx mori* cocoons at 60^0C and 70ºC for 120 minutes.

The highest mean weight loss in *B. mori* cocoons with papain at 60 ºC, 120 minute was 0.08 ± 0.008g and this was significantly different from all the other treatments. This was followed by bromelain, sodium bicarbonate and pineapple extract.

Table 4.3d Mean weight loss (± SE) of *B. mori* cocoons at 60 ºC and 70 ºC for 120 minutes.

Treatment	60^0C, 120 min	70^0C, 120 min
Water	0.03 ± 0.003^{aCD}	0.03 ± 0.003^{aB}
Pineapple	0.05 ± 0.005^{aBC}	0.03 ± 0.001^{bB}
$NaHco_3$	0.04 ± 0.009^{aBC}	0.07 ± 0.007^{aA}
Bromelain	0.06 ± 0.004^{aB}	0.04 ± 0.004^{bB}
Ripe papaya	0.01 ± 0.002^{aE}	0.04 ± 0.010^{aB}
Raw papaya	0.02 ± 0.003^{aDE}	0.03 ± 0.003^{aB}
Papain	0.08 ± 0.005^{aA}	0.02 ± 0.002^{aB}

Means within a column followed by different upper case and each pair within the row followed by different lower case letter(s) are significantly different (p=0.05 SNK)

The lowest mean weight loss was recorded with ripe papaya; however this was not significantly different from raw papaya. Higher temperature had no effect on the mean weight loss except with bromelain and pineapple extract where lower mean weight loss was recorded. At 70 ºC, 120 minutes, sodium bicarbonate gave the highest significantly different mean weight loss. No significant difference in the mean weight loss from the cocoons was observed for all the other treatments (Table 4.3d).

CHAPTER FIVE

5.0 DISCUSSION

Result from the study showed that the mean weight loss in *G. postica* cocoons with commercial papain and bromelain treatments were significantly high at 30 and 60 minutes. Papain gave the highest mean weight loss at 60 °C, 120 minutes. These findings are in agreement with the results reported by Laishram *et al*., (2003) who determined the optimum temperature for proteinase activity to be 60°C. On the other hand, bromelain gave the highest weight loss at 70° C, 60 minutes. This temperature is higher than the 60°C which has been reported by Laishram *et al.* (2003) as the optimum temperature for the protease activity. This was probably due to increase in enzyme bromelain activity as the temperature increased. Treatment with the crude fruit extracts yielded a lower mean weight loss from the cocoons than the commercial protease's. During the treatment of cocoons, ripe and raw papaya extract were used but only the ripe pineapple extract was used as it's protainase activity is about 30 % higher than the extract from the unripe pineapple. (Laishram *et al*., 2003). The pineapple extract gave the highest mean weight loss at 60°C and 90 minutes but this was not different from the weight loss which resulted from the water treatment in *G. postica* cocoons. It also was observed that sodium bicarbonate gave a higher weight loss than the pineapple extract treatment in all the durations. Raw papaya extract gave a high mean weight loss at 60 °C, 90 minutes but this was not different from the weight loss which resulted from the water and sodium bicarbonate treatments. Ripe

papaya extract gave the lowest mean weight loss in *G. postica* cocoons at 60 °C and 70 °C for all durations.

Protainase activity was noted to increase with the duration; the increase in activity was however more at 60^0 C than it was at 70 ^{0}C. similar observations have been reported for oak tasar silk cocoons (Lashram *et al.,* 2003). The raw papaya extract treatment gave the highest mean weight loss of 0.41g with *G. postica* cocoons at a temperature of 60°C at 90 minutes. However this was not significantly different from weight loss for the other treatments. This weight loss was higher than the corresponding mean weight loss obtained from the ripe papaya and water treatments, thus indicating a higher concentration of proteinase in the raw papaya extract (Murthy and Natarajan, 1992). On the other hand, water treatment gave a higher weight loss than the ripe papaya treatment at a higher temperature. The explanation here could be that the liquor ratio in the extracts was 1:1 for water and the papaya extract and it is observed that sericin hydrolysis at higher temperature in water (Gulrajani, 1992). Consequently, there were more water molecules in the distilled water than there were in the papaya extracts. However, no significant difference was observed between the weight loss obtained from the ripe papaya extract treatment and that which was obtained from the water treatment.

Bromelain treatment gave different results from the other treatments with the highest mean weight loss of 0.42g in *G. postica* observed at a temperature of 70°C at 60 minutes. This weight loss was significantly different from the weight loss of all the other treatments at 60°C at the same duration. Bromelain treatment resulted to recording a higher cocoon weight loss than that which was obtained from both

water and sodium bicarbonate treatments. However, the only significant difference in weight loss was recorded at 30 and 60 minutes at both 60° C and 70° C indicating that a longer duration reduced the effect of the enzyme (Rowan and Battle, 1994).

Papain treatment gave the highest weight loss of *A. mimosae* cocoons at a temperature of 60°C and a duration of 120 minutes and this was significantly different from the weight loss from the other treatments. Bromelain gave the second best results of cocoon weight loss at 60º C in 90 minutes but this was not significantly different from the weight loss from the other treatments. However, the raw and ripe papaya, pineapple extract and sodium bicarbonate treatments required a temperature of 70º C to produce their best results. According to Greenberg (1955), the activities of protease decreases with increase in temperature above the optimum temperature as enzymes are susceptible to high temperatures value outside their optimum range because they are denatured. *Argema. mimosae* weight losses were higher in higher temperatures and this may be explained by the fact that some sericin may be hydrolysed in water at a higher temperature considering that the liquor ratio was 1:1 for water and the papaya extract, while water and sodium bicarbonate hydrolyses sericin at a higher temperature of 98^0 C (Hisao, 1994).

Bombyx mori cocoons gave the higher mean weight losses at both 60 °C and 70^0C with papain treatment which was only significantly different from the weight loss obtained from the sodium bicarbonate treatment at the same duration and

temperatures. However, these weight losses were not significantly different from the water treatment. With bromelain treatment *Bombyx mori* cocoons gave the highest weight loss of 0.06g occurred at 120 minutes, 60^0 C. This weight loss was higher than 0.03g which resulted from the water treatment at the same duration. However, at 120 minutes and 70^0 C, sodium bicarbonate gave higher weight losses than all the other treatments. This is because sodium bicarbonate hydrolysed sericin at high temperature and conventionally degumming with sodium bicarbonate involves boiling.The disadvantage of this is the high temperature and the high pH which may result in the damage of fibroin (Hisao, 1994). Ripe and raw papaya extract treatments gave their highest weight losses at 70 ^{0}C in 120 and 90 minute respectively. Here, the effect of high temperature and water in the extract can not be overruled. It also was observed that sodium bicarbonate treatment gave a higher weight loss than the raw and ripe papaya extract treatments at 70 ^{0}C (Dupaigne, 1973).

Research work has been carried out on the benefit of using proteolytic enzyme in the degumming of *Bombyx mori* silk moth cocoons (Gulrajan, 1992). However, the East African silk moths have not been extensively researched on. Apart from being inexpensive, the degumming of cocoons process which is carried out in a single bath without any pretreatment or post treatment results in the saving of time and energy. The results obtained from this study showed that plant proteases can be used in the degumming of *G. postica* and *B. mori cocoons* at a temperature of 60ºC and at a shorter duration, thus reducing the processing cost of cocoons. However, the use of plant protease did not give good results for *A. mimosae*

cocoons. Papain was observed to be the best agent for degumming *G. postica* and *B. mori* cocoons as it gave the highest weight losses and required low temperature and a shorter duration for the cocoon to be soft enough to enable the removal of floss to facilitate spinning. In addition, the enzymatic method of sericin removal has been reported to have produced better results; particularly in terms of weight loss, elasticity and resilience than the conventional soap, soda or a mixture of soap and soda methods (Kim and Nahm, 1987). Enzyme degumming has the following advantages over the convectional degumming methods. It has a specific reaction thereby it may give a minimum damage to the fibroin, It has the lesser risk of over degumming than the convectional methods and Weight loss may be modified by adjusting the concentration of enzymes, the reaction time and temperature
(Shukla *et al* 1992).

The degumming of cocoons can also be done through an economically acceptable and environmentally friendly production method. An alternative method for the production of silk using sustainable method is more obvious with the imminent climatic change.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMEDATIONS

6.1 Conclusions

From the results obtained from this study the following conclusions can be made

a) Different treatments resulted to having significant weight losses in different silk moth cocoons. When *G. postica* cocoons were treated with the pineapple fruit extract, the highest weight loss was obtained at 60° C in a duration of 90 minutes followed by *A. mimosae*, at 70°C in a duration of 60 minutes. This was followed by *B. mori* at 120 minutes and a temperature of 60° C. Thus, it can be concluded that pineapple extract has different degumming effects on the various silkworm cocoons.

b) Raw papaya extract treatments on cocoons of the three species of silk moths also gave different weight losses. *G. postica* cocoons had a higher cocoon weight loss at 60° C for a duration of 90 minutes followed by *A. mimosae* at 70° C in a duration of 30 minutes and then *B. mori* at 90 minutes and a temperature of 70° C.

c) Treatment of *G. postica* cocoons with raw papaya extract gave the highest cocoon weight loss at 70° C in a duration of 120 minutes followed by *A. mimosae* at 70° C in a duration of 60 minutes. For *B. mori*, the highest cocoon weight loss occurred at 120 minutes at a temperature of 70° C. Therefore, cocoons of the three species of silk moths require a temperature of 70º C to give the best results with the ripe papaya extract treatment.

d) Bromalein treatment required a less time of 60 minutes to give the highest cocoon weight loss at a temperature of 70° C in *G. postica*, but the highest *A. mimosae* cocoon weight loss was recorded at a temperature of 60° C but it required a time of 90 minutes. *Bombyx mori* had higher cocoon weight losses at a temperature of 60° C, but required a time of 120 minutes. Therefore, Bromelain seemed to be more effective in all the three silk moth cocoons at a temperature of 60ºC.

e) Papain treatment gave the best results with the *G. postica*, *A. mimosae* and *B. mori* cocoons. However a longer duration of 120 minutes and a temperature of 60 ºC were required for *G. postica* and *A. mimosae* to give the highest cocoon weight loss. *B. mori* gave the highest cocoon weight loss of 0.12 ± 0.007g at a temperature of 70° C and a duration of 70 minutes

6.2 Recommendations

The commercial treatment agents (bromelain and papain) gave a higher mean weight loss. However, at a cost of 1.5 dollars per gram, they are expensive and in addition they are not readily available in the local market. Therefore raw papaya extract is recommended as it gave a higher mean weight loss at a lower temperature of 60°C for *G. postica*; but with *A. mimosae* and *B. mori,* a higher temperature of 70° C will be required. The degumming process was carried out in a single bath without any pretreatment or post treatment resulting in saving time and energy as it requires low temperature and a shorter duration for the removal of

sericin to facilitate the making of floss for spinning when compared with the conventional methods. The results also showed that plant protease can be used in the degumming of *G. postica*, *B. mori* and *A. mimosae* cocoons at a lower temperature and a shorter duration, thus reducing the cost of processing the cocoons. It was not possible during this study to investigate all aspects of the interactions between temperature, treatments and the quality of the silk produced. It is therefore recommended that the following aspects be investigated in detail to support the effectiveness of the enzymatic treatment for degumming of cocoons of silk moths.

1. There is need to study the effect of interaction between temperature and proteinase on the quality of *G. postica*, *A. mimosae* and *B. mori* silk.
2. There is need to evaluate the effect of interaction of different proteinases with sodium bicarbonate and their effect on the quality of silk.
3. There is need to study the properties of sericin protein from wild silk moth and its potential value in cosmetic industries.

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APPENDICES

Appendix I ANOVA table for mean weight loss from *Gonometa postica* cocoons

a)Gonometa postica at Time=30, Temp=60 ºC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.15396297	0.02566050	23.03	<.0001*
Error	28	0.03119720	0.00111419		
Corrected Total	34	0.18516017			

b)Gonometa postica at Time=30, Temp=70 ºC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.20015514	0.03335919	13.44	<.0001*
Error	28	0.06947440	0.00248123		
Corrected Total	34	0.26962954			

c)Gonometa postica at Time=60, Temp=60 ºC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.38524949	0.06420825	21.21	<.0001*
Error	28	0.08476240	0.00302723		
Corrected Total	34	0.47001189			

d)Gonometa postica in Time=60, Temp=70 ºC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.45166937	0.07527823	7.49	<.0001*
Error	28	0.28130520	0.01004661		
Corrected Total	34	0.73297457			

e)Gonometa postica in Time=90, Temp=60 ºC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.30777529	0.05129588	1.43	0.2390*
Error	27	0.96710257	0.03581861		
Corrected Total	33	1.27487786			

f)Gonometa postica in Time=90, Temp=70 ºC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.23727920	0.03954653	6.68	0.0002*
Error	28	0.16577440	0.00592051		
Corrected Total	34	0.40305360			

g)Gonometa postica in Time=120, Temp=60 ºC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.39795274	0.06632546	16.18	<.0001*
Error	28	0.11475640	0.00409844		
Corrected Total	34	0.51270914			

h)Gonometa postica in Time=120 Temp=70 ºC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.34918789	0.05819798	5.20	0.0011*
Error	28	0.31332880	0.01119031		
Corrected Total	34	0.66251669			

Appendix II ANOVA table for mean weight loss from *Argema mimosae* cocoons

a) *Argema mimosae* in Time=30, Temp=60 ºC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.00248320	0.00041387	1.44	0.2339*
Error	28	0.00803120	0.00028683		
Corrected Total	34	0.01051440			

b) *Argema mimosae* in Time=30 Temp=70 ºC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.00424109	0.00070685	1.83	0.1293*
Error	28	0.01081720	0.00038633		
Corrected Total	34	0.01505829			

c) *Argema mimosae* in Time=60 Temp=60 ºC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.00300434	0.00050072	1.85	0.1257*
Error	28	0.00758680	0.00027096		
Corrected Total	34	0.01059114			

d) *Argema mimosae* in Time=60, Temp=70 ºC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.01596314	0.00266052	0.57	0.7475
Error	28	0.12974000	0.00463357		
Corrected Total	34	0.14570314			

e) *Argema mimosae* in Time=90 Temp=60 ºC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.01662634	0.00277106	6.54	0.0002
Error	28	0.01185640	0.00042344		
Corrected Total	34	0.02848274			

f) *Argema mimosae* in Time=90 Temp=70 ºC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.00158217	0.00026370	0.50	0.8012
Error	28	0.01470080	0.00052503		
Corrected Total	34	0.01628297			

g) *Argema mimosae* in Time=120 Temp=60 ºC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	15.02577097	2.50429516	1.01	0.4369
Error	28	69.21333200	2.47190471		
Corrected Total	34	84.23910297			

h) *Argema mimosae* in Time=120 Temp=70 ºC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.02424560	0.00404093	3.38	0.0124
Error	28	0.03349640	0.00119630		
Corrected Total	34	0.05774200			

Appendix III ANOVA table for mean weight loss from *Bombyx mori* cocoons

a) *Bombyx mori* in Time=30 Temp=60 ºC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.02049714	0.00341619	69.60	<.0001
Error	28	0.00137440	0.00004909		
Corrected Total	34	0.02187154			

b) Bombyx mori

Time=30 Temp=70 ºC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.01553657	0.00258943	17.27	<.0001
Error	28	0.00419800	0.00014993		
Corrected Total	34	0.01973457			

c)Bombyx mori

Time=60 Temp=60 ºC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.02645709	0.00440951	30.22	<.0001
Error	28	0.00408560	0.00014591		
Corrected Total	34	0.03054269			

c) Bombyx mori

Time=60 Temp=70 ºC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.03376080	0.00562680	55.72	<.0001
Error	28	0.00282760	0.00010099		
Corrected Total	34	0.03658840			

d) Bombyx mori

Time=90 Temp=60 ºC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.01559897	0.00259983	25.09	<.0001
Error	28	0.00290160	0.00010363		
Corrected Total	34	0.01850057			

e) Bombyx mori

Time=90 Temp=70 ºC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.01842097	0.00307016	1.97	0.1040
Error	28	0.04362920	0.00155819		
Corrected Total	34	0.06205017			

g) Bombyx mori

Time=120 Temp=60 ºC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.01430674	0.00238446	19.98	<.0001
Error	28	0.00334200	0.00011936		
Corrected Total	34	0.01764874			

h) Bombyx mori

Time=120 Temp=70 ºC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.00720297	0.00120050	8.49	<.0001
Error	28	0.00396120	0.00014147		
Corrected Total	34	0.01116417			