Screening the Antiglycation Properties of *Orthosiphon aristatus (Cat's whisker)* and *Thunbergia laurifolia* (Laurel Vine Tea) in Model System

By

Shriya Gorowara

5335112

A special project submitted to School of Biotechnology, Assumption University of Thailand in part fulfillment of the Degree of Bachelor of Science in Biotechnology

2014

Screening the Antiglycation Properties of *Orthosiphon aristatus (Cat's whisker)* and *Thunbergia laurifolia* (Laurel Vine Tea) in Model System

By

Shriya Gorowara

5335112

A special project submitted to School of Biotechnology, Assumption University of Thailand in part fulfillment of the Degree of Bachelor of Science in Biotechnology

2014

By	Miss Shriya Gorowara
Advisor	A.Roungdao Klinjapo
Co-Advisor	Dr. Pornpong Suttnirak
Level of study	Bachelor of Science
Faculty	Biotechnology
Major	Food Technology
Academic Year	2014

A.Roungdao Klinjapo

(Advisor)

Dr. Pornpong Suthirak

(Co-Advisor)

ACKNOWLEDGEMENT

This project was completed with kind assistance from many contributors. Therefore, I would like to take this special opportunity to thank those who helped prepare and support this project.

First of all, I would like to express my sincere gratitude to my advisor, A. Roungdao Klinjapo for her aspiring and excellent guidance, encouragement and friendly advice by contributing her professional knowledge particularly throughout this project. It would not have been completed or written without her.

Besides the advisor, I would like thank the project committee members which includes Dr. Pornpong Suthirak (Co-advisor) and Dr. Atittaya Tandhanskul, who was always willing to help and give their best suggestions. Their innovative ideas and supporting act improved the project to be a better one.

Ms. Shriya Gorowara

ID. 5335112

Screening the antiglycation properties of Cat's whisker tea (*Orthosiphon aristatus*) and Laurel clock vine tea (*Thunbergia laurifolia*) in model systems

---

ABSTRACT

Glucose (Glu), Fructose (Fru), Glycine (Gly), and Lysine (Lys) were prepared at 0.5 M using phosphate buffer pH 7.39 as the solvent. Four model systems were prepared as Fructose-Lysine and Glucose-Lysine at the ratio 1:2, while Fructose-Glycine and Glucose-Glycine were prepared at the ratio 1:1. Cat's whisker tea (C) and Laurel Vine Tea (L) were prepared at 0.5, 0.75 and 1.0% (w/v) by steeping dried tea in 100 ml of 90°C water for 15 minutes. Brewed tea was added into the model system at the ratio of 1:1, and then all models were heated at 60°C for 7 hours. Samples were sampling every 1 hour to measure the browning at OD 420 nm compared with control models. As the results, the best anti-glycation property was 1.0% C and 0.5% L for Fructose-Glycine model, 0.5% C for Glucose-Glycine model, 1.0% C and 1.0% L for Fructose-Lysine model, and 0.75% C and 0.5% L for Glucose-Lys model. All the best condition of each model systems were investigated the 5-hydroxymethylfurfural (HMF) content and sugar content compared with control using HPLC analysis. The results concluded that the best condition to retard glycation was 0.5% C for both Fructose-Glycine and Glucose-Glycine model, 1.0% L for Fructose-Lysine model, and 0.75% L for Glucose-Lysine model.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Nowadays, there are lots of natural products in the market which are beneficial for health. The requirement of these natural healthy products is increased in the market every year. Even though, many kinds of healthy product was not analyzed systematically how much it effect to the human body. Therefore, there is a research and development of the natural product which might provide health benefits.

Millions of people in the world are suffering from diabetes which is also known as the metabolic diseases. Diabetes is a disease in which blood glucose, or sugar, levels are too high. It's either because the body doesn't make insulin or because the cells do not respond to the insulin produced. Insulin is a hormone that helps the glucose gets into the cells to give them energy. Many research papers revealed that diabetes mellitus was related with the development of glycation or Maillard reaction. Glycation is a non-enzymatic reaction of protein by glucose. Through a series of complex reactions, advanced glycation end products (AGEs) are generated. Glycation also occurs between lipids and glucose, results in glycation of phospholipids in cell membrane. It has been reported that dietary advanced glycation end products (dAGEs) are known to contribute to increased oxidant stress and inflammation, which are linked to the recent epidemics of diabetes and cardiovascular disease. This process is of particular concern for diabetics, who already suffer from the effects of poor glucose control. In fact, elevated levels of AGEs contribute to a number of diabetes-related complications, including neuropathy, retinal disease, and kidney failure.

As the focus on diabetes, herbal tea drinking is a good method to help the inhibition of glycation development. Laurel clock vine (*Thumbergia laurifolia*) and Cat's Whisker (*Orthosiphon aristatus*) are two herbal teas that were claimed to contain antioxidant and anti-inflammatory properties. Laurel clock vine or Ran-jued in Thai language is a vigorous, perennial, climbing vine. Most of antidote mechanism of Ran-jued is anti-oxidative activity and it has active ingredients that can neutralize the toxins before they will react with cells. The tea has been claimed to be able to detoxify the harmful effects of drugs, alcohol and cigarettes. Active compounds that isolated from the leaves of Laurel Clock Vine are consisting of iridoid glucosides, grandifloric acid, glucopyranosides, and derivatives of apigenin, delphinidin derivatives, and phenolic acids of chlorogenic, caffeic, gallic, and protocatechuic. Cat's Whisker (*Orthosiphon aristatus*) is a member of the mint family of plants. It has long been used in South-East Asia for its medicinal properties. Traditionally, it

has been used to reduce cellulite, and as an antispasmodic, as well as for the internal organs. It can reduce blood sugar levels and there is hope that it will be useful for diabetics who do not depend on insulin. It also has antioxidant and anti-inflammatory actions and research suggests that it also useful for reducing high blood pressure and has antimicrobial and anti-fungal properties.

Thus, the aim of this senior project is to study the effect of Laurel Clock Vine and Cat's Whisker tea that are known to inhibit the glycation development which can relate to the decreasing of blood sugar level.

OBJECTIVES

1. To study the anti-glycation properties of Laurel clock vine (*Thunbergia laurifolia*) and Cat's Whisker (*Orthosiphon aristatus*) tea

2. Study the effect of the concentration of Laurel clock vine and Cat's Whisker tea on the development of glycation

LITERATURE REVIEW

1. GLYCATION

Glycation is a reaction that takes place when simple sugar molecules, such as fructose or glucose, become attached to proteins or lipid fats without the moderation of an enzyme. This results in the formation of rogue molecules known as advanced glycation end products (AGEs). This process, also known as non-enzymatic glycosylation, is normally governed by enzymatic activity, which is necessary to regulate the metabolic functioning of molecules. The lack of this catalyst deters the normal glycosylation of sugars to produced needed energy, however, and since it disrupts normal metabolic pathways and advances the circulation of AGEs, it can promote certain health risks.

Exogenous glycation, one form that occurs outside the body, are responsible for allowing foods to brown during cooking. This type is also known as the *Maillard reaction*, where sugars react with fats or proteins while exposed to high temperatures. In addition, exogenous AGEs are sometimes added to certain foods to enhance color and flavor, including baked goods, dark colas, and coffee.

Endogenous glycation, which occurs in the body, is associated with increased oxidative damage. AGEs and their by-products are linked to many age-related diseases, including Alzheimer's. This process is of particular concern for diabetics, who already suffer from the effects of poor glucose control. In fact, elevated levels of AGEs contribute to a number of diabetes-related complications, including neuropathy, retinal disease, and kidney failure. [1] [2]

1.1 Glycation Reactions

This reaction is subdivided into three main stages: early, intermediate, and late stage. In the early stage, glucose (or other reducing sugars) reacts with a free amino group of biological amines, to form an unstable aldimine compound or the Shiff base product. Then through acid-base catalysis, this compound undergoes a rearrangement to a more stable early glycation product known as Amadori Rearrangement Product (ARP).

In the intermediate stage, via dehydratation, oxidation and other chemical reactions, the Amadori product degrades to a variety of reactive dicarbonyl compounds such as glyoxal,

methylglyoxal, and deoxyglucosones which, being much more reactive than the initial sugars, act as propagators of the reaction, again reacting with free amino groups of biomolecules.

In the late stage of the glycation process through oxidation, dehydration and cyclization reactions, irreversible compounds called Advanced Glycation End Products (AGEs) are formed. The AGEs are yellow-brown substances, often fluorescent and insoluble adducts that accumulate on long-lived proteins thus compromising their physiological functions. [3]

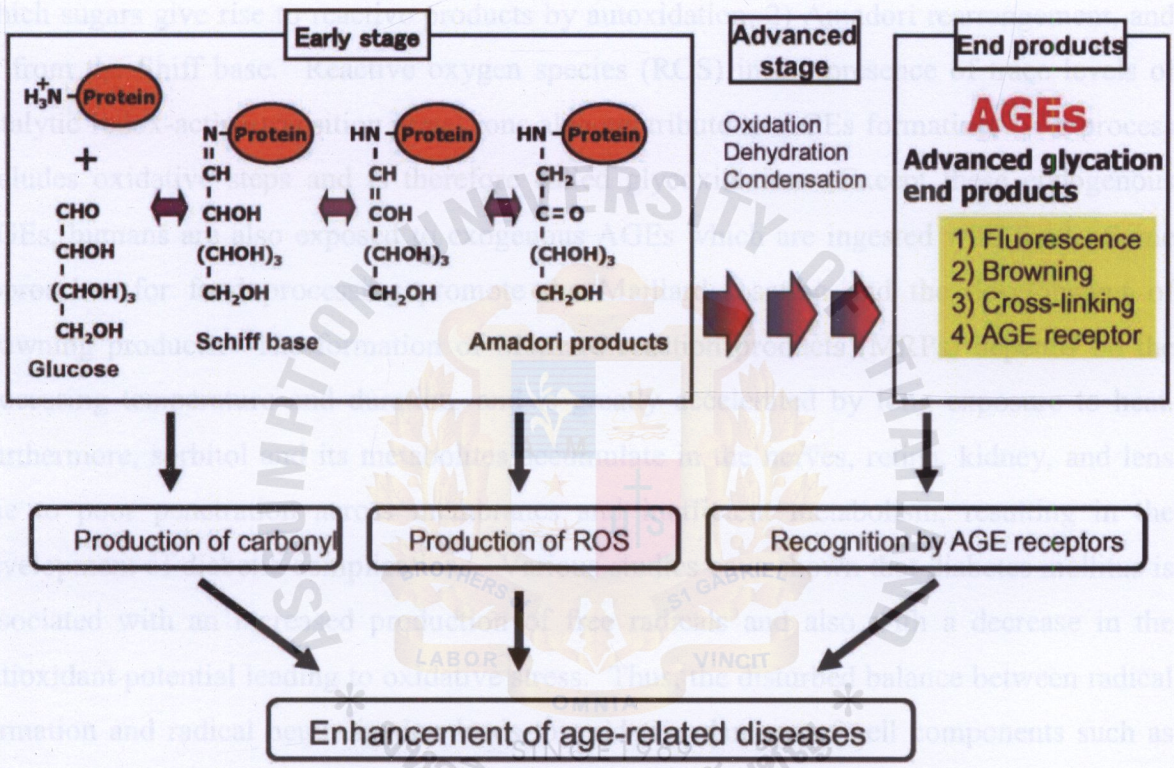


Figure 1: Glycation reaction or Maillard reaction [4]

1.2 Advanced Glycation End Products (AGEs)

The formation of AGEs progressively increases with normal aging and age-dependent. The formation of AGEs is a part of normal metabolism, but if excessively high levels of AGEs are reached in tissues and the circulation they can become pathogenic. The pathologic effects of AGEs are related to their ability to promote oxidative stress and inflammation by binding with cell surface receptors or cross-linking with body proteins, altering their structure and.[5]

AGEs have been shown to accumulate in human cartilage, skin collagen and pericardial fluid. Long-lived proteins such as lens crystallins and especially collagens contain numerous lysine, hydroxylysine and arginine residues, have a slow turn over, and are prone to age-related accumulation of glycation damage. Besides accumulation during healthy aging, AGEs are formed at accelerated rates in diabetes. They are markers and also important causative factors for the pathogenesis of diabetes, cataracts, atherosclerosis, diabetic nephropathy, and neurodegenerative diseases, including Alzheimer's disease.

Three routes have been proposed for AGEs formation: 1) autoxidative pathway in which sugars give rise to reactive products by autoxidation, 2) Amadori rearrangement, and 3) from the Shiff base. Reactive oxygen species (ROS) in the presence of trace levels of catalytic redox-active transition metal ions also contribute to AGEs formation. The process includes oxidative steps and is therefore called glycoxidation. Except these endogenous AGEs, humans are also exposed to exogenous AGEs which are ingested with food. Some approaches for food processing promote the Maillard reaction and the development of browning products. The formation of Maillard reaction products (MRPs) depends on the processing temperature and duration, and is greatly accelerated by long exposure to heat. Furthermore, sorbitol and its metabolites accumulate in the nerves, retina, kidney, and lens due to poor penetration across membranes and inefficient metabolism, resulting in the development of diabetic complications. Various studies have shown that diabetes mellitus is associated with an increased production of free radicals and also with a decrease in the antioxidant potential leading to oxidative stress. Thus, the disturbed balance between radical formation and radical neutralization leads to oxidative damage of cell components such as proteins, lipids, and nucleic acids.[6] [7]

1.3 Factors affected glycation

Many factors influenced the velocity of glycation or Maillard reaction such as temperature, water activity (Aw), reactant source and concentration, type and ratio of reducing sugar, amino compound, pH, and food composition. [8]

1.3.1 *Amino acids*

Amino acids are organic compounds that combine to form proteins. Amino acids and proteins are the building blocks of life. When proteins are digested or broken down, amino acids are left. The human body uses amino acids to make proteins to help the body: to break

down food, to grow, to repair body tissue, and to perform many other body functions. Amino acids can also be used as a source of energy by the body.

Amino acids have a two-carbon bond. One of the carbon s is part of a group called the carboxyl group (COO-). A carboxyl group is made up of one carbon (C) and two oxygen (O) atoms. That carboxyl group has a negative charge, since it is a carboxylic acid (-COOH) that has lost its hydrogen (H) atom. What is left – the carboxyl group – is called a conjugate base. The second carbon is connected to the amino group. Amino means there is an NH_2 group bonded to the carbon atom. In the image, you see a "+" and a "–". Those positive and negative signs are there because, in amino acids, one hydrogen atom moves to the other end of the molecule. An extra "H" gives you a positive charge.

Types of amino acid

(i) *Essential amino acids*

Essential amino acids cannot be made by the body. As a result, they must come from food. There are nine essential amino acids that are, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine.

(ii) *Nonessential amino acids*

"Nonessential" means that our bodies produce an amino acid, even if we don't get it from the food we eat. They include: alanine, asparagine, aspartic acid, and glutamic acid.

(iii) *Conditional amino acids*

Conditional amino acids are usually not essential, except in times of illness and stress. They include: arginine, cysteine, glutamine, tyrosine, glycine, ornithine, proline, and serine.[9]

1.3.1.1 *Glycine*

Glycine is a sweet-tasting crystalline nonessential amino acid, $C_2H_5NO_2$ that is the simplest and indeed is the smallest amino acid found in protein. Glycine is a colorless, sweet-tasting crystalline solid. It is unique among the proteinogenic amino acids in that it is not chiral. It can fit into hydrophilic or hydrophobic environments, due to its minimal side chain of only one hydrogen atom. In the free state, glycine participates in several important reactions, including the biosynthesis of heme, an important constituent of hemoglobin, and the biosyntheses of serine (another amino acid), purines (constituents of genetic material), and glutathione (a coenzyme). Therefore, glycine is not essential amino acid to the human diet, as it is biosynthesized in the body from Serine, which is in turn derived from 3-phosphoglycerate. Defects of glycine metabolism are very rare. The amino acid is not

essential to the diet since it can be made from other substances in the body. In most organisms, the enzyme "Serine hydroxyl methyltransferase" catalyzes this transformation via the cofactor pyridoxal phosphate[10]:

Serine + tetrahydrofolate → glycine + N^5,N^{10}-Methylene tetrahydrofolate + H_2O

In term of health, glycine is used for treating schizophrenia, stroke, benign prostatic hyperplasia (BPH), and some rare inherited metabolic disorders. It is also used to protect kidneys from the harmful side effects of certain drugs used after organ transplantation as well as the liver from harmful effects of alcohol. Other uses include cancer prevention and memory enhancement. Some people apply glycine directly to the skin to treat leg ulcers and heal other wounds.

The body uses glycine to make proteins. Glycine is also involved in the transmission of chemical signals in the brain, so there is interest in trying it for schizophrenia and improving memory. Some researchers think glycine may have a role in cancer prevention because it seems to interfere with the blood supply needed by certain tumors.[11]

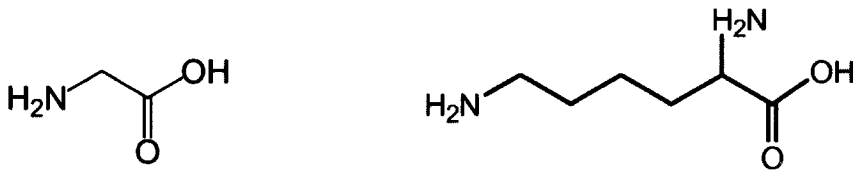
1.3.1.2 *Lysine*

Lysine, or L-lysine, is an essential amino acid of special nutritional importance, since it is the limiting amino acid in many cereals. It can be synthesized on a commercial scale, and when added to bread, rice, or cereal-based animal feeds. Thus, it improves the nutritional value of the protein. Not all of the lysine in proteins is biologically available, since some is linked through its side-chain amino group, either to sugars (as Maillard reaction), or to other amino acids. These linkages are not hydrolyzed by digestive enzymes, and so the lysine cannot be absorbed. Available lysine is that proportion of the protein-bound lysine in which the side-chain amino group is free, so that it can be absorbed after digestion of the protein.[12]

Lysine is important for proper growth and it plays an essential role in the production of carnitine, a nutrient responsible for converting fatty acids into energy and helping to lower cholesterol. Lysine appears to help the body absorb calcium, and it plays an important role in the formation of collagen, a substance important for bones and connective tissues including skin, tendon, and cartilage.

Foods rich in protein are good sources of lysine. Protein-rich foods include meat (specifically red meat, pork, and poultry), cheese (particularly parmesan), certain fish (such as cod and sardines), nuts, eggs, soybeans (particularly tofu, isolated soy protein, and defatted

soybean flour), and fenugreek seed. Brewer's yeast, beans and other legumes, and dairy products also contain lysine. Many nuts also contain lysine along with arginine (lysine counteracts some of the effects of arginine). [13]



(a) (b)

Figure 2: Structural formula of glycine (a) and lysine (b)

1.3.2 *Sugars*

1.3.2.1 *Glucose*

Glucose is a monosaccharide sugar with the molecular formula $C_6H_{12}O_6$. It is also known as dextrose or grape sugar. With 6 carbon atoms, it is classed as a hexose, a sub-category of monosaccharides. The α-D-glucose is one of the 16 aldose stereoisomers. D-isomer occurs widely in nature, but the L-isomer does not. Glucose is made during photosynthesis from water and carbon dioxide, using energy from sunlight. The reverse of the photosynthesis reaction, which releases this energy, is a very important source of power for cellular respiration. Glucose may be stored in plants as the polymers starch and cellulose.[14]

Glucose in free or combined form is not only the most common of the sugars but is probably the most abundant organic compound in nature. It is found in considerable concentrations in grapes, figs, and other sweet fruits and in honey. In lesser concentrations, it occurs in the animal body fluids, for example, in blood and lymph. Urine of diabetic patents usually contains 3–5%.[15]

Glucose, as opposed to other hexose sugars, is widely used in living organisms. Glucose has a lower tendency than other hexose sugars to react non-specifically with the amino groups of proteins. This reaction – glycation – impairs or destroys the function of many enzymes. The low rate of glycation is due to glucose's stability in the cyclic isomer state, which is less reactive than other hexose isomers. Nevertheless, many of the long-term complications of diabetes (e.g., blindness, renal failure, and peripheral neuropathy) are

probably due to the glycation of proteins or lipids. In contrast, enzyme-regulated addition of glucose by glycosylation is essential to the function of many proteins. Another possible explanation for the widespread use of glucose is that it is the most conformational stable compared to other possibilities.

In plants and some prokaryotes, glucose is a product of photosynthesis. In animals and fungi, glucose results from the breakdown of glycogen, a process known as glycogenolysis. In plants the breakdown substrate is starch. In animals, glucose is synthesized in the liver and kidneys from non-carbohydrate intermediates, such as pyruvate, lactate and glycerol, by a process known as gluconeogenesis. In some deep-sea bacteria, glucose is produced by chemosynthesis.

For commercially purpose, glucose is produced via the enzymatic hydrolysis of starch. Many crops can be used as the source of starch. Maize, rice, wheat, cassava, corn husk, and sago are all used in various parts of the world. Most commercial glucose occurs as a component of invert sugar, a roughly 1:1 mixture of glucose and fructose. In principle, cellulose could be hydrolyzed to glucose, but this process is not yet commercially practical. [16]

1.3.2.2 *Fructose*

Fructose, or fruit sugar, is one of a simple ketone monosaccharides found in many plants, where it is often bonded to glucose to form the disaccharide sucrose. It is one of the three dietary monosaccharides, along with glucose and galactose. Pure, dry fructose is a very sweet, white, odorless, crystalline solid and is the most water-soluble of all the sugars. Fructose is found in honey, tree and vine fruits, flowers, berries, and most root vegetables.

Commercially, fructose is frequently derived from sugar cane, sugar beets, and corn. Crystalline fructose is the monosaccharide, dried, ground, and of high purity. High-fructose corn syrup (HFCS) is a mixture of glucose and fructose as monosaccharaides. Sucrose is a compound with one molecule of glucose covalently linked to one molecule of fructose. All forms of fructose, including fruits and juices, are commonly added to foods and drinks for palatability and taste enhancement, and for browning of some foods, such as baked goods.

Fructose may be anaerobically fermented by yeast or bacteria. Yeast enzymes convert sugar (glucose, or fructose) to ethanol and carbon dioxide. The carbon dioxide released during fermentation will remain dissolved in water, where it will reach equilibrium

with carbonic acid, unless the fermentation chamber is left open to the air. The dissolved carbon dioxide and carbonic acid produce the carbonation in bottled fermented beverages.

Fructose undergoes the Maillard reaction, non-enzymatic browning, with amino acids. Because fructose exists to a greater extent in the open-chain form than glucose, the initial stages of the Maillard reaction occur more rapidly than with glucose. Therefore, fructose has potential to contribute to changes in food palatability, as well as other nutritional effects, such as excessive browning, volume and tenderness reduction during cake preparation, and formation of mutagenic compounds. Fructose readily dehydrates to give hydroxyl methylfurfural (HMF). This process, in the future, may become part of a low-cost, carbon-neutral system to produce replacements for petrol and diesel from plants.

Fructose absorption occurs in the small intestine via the GLUT-5 (fructose only) transporter, and the GLUT2 transporter, for which it competes with glucose and galactose. Over-consumption of fructose, inhibition of GLUT2 by other phytochemicals, such as flavonoids, or other issues, may result in delivery of unabsorbed fructose into the large intestine, where it may provide nutrients for the existing gut flora.

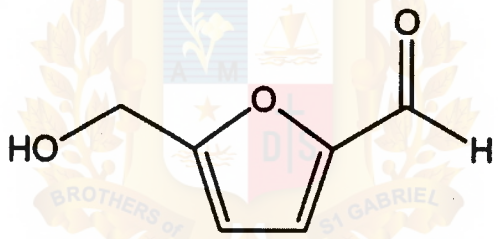


Figure 3: Structure of hydroxyl methylfurfural (HMF)

Excess fructose consumption has been hypothesized to be a cause of insulin resistance, obesity, elevated LDL cholesterol and triglycerides, leading to metabolic syndrome. In preliminary research, fructose consumption was correlated with obesity. To compare with consumption of high glucose beverages, drinking high-fructose beverages with meals results in lower circulating insulin and leptin levels, and higher ghrelin levels after the meal. Since leptin and insulin decrease appetite and ghrelin increases appetite, some researchers suspect that eating large amounts of fructose increases the likelihood of weight gain. Excessive fructose consumption may contribute to the development of non-alcoholic fatty liver disease. A preliminary human study indicated that fructose may not influence

metabolic activity or blood flow in brain regions regulating satiety (fullness), and so may promote overeating. [17]

2. Anti – Glycation

Anti-Glycation (or anti-AGEs) represents a new prophylactic effect against glycosylation or glycation. Anti-Glycation is becoming an important therapeutic defensive benefit against endogenous and exogenous aging factors leading to glycation. Anti-Glycation is also the inhibition of the chemical reaction between sugar radicals and proteins leading to glycation. Anti-AGE drugs are being intensively studied. Aminoguanidine was the first drug designed to inhibit glycation reactions by inhibiting the conversion of early products to AGEs. Animal studies proved that aminoguanidine is beneficial for many diabetes-related complications. Additional drugs that inhibit AGE formation or disrupt already formed AGEs (e.g., AGE-breakers) are also under active investigation.

Aminoguanidine, has also been shown to prevent retinopathy in diabetic animals. It is also known that AGEs accumulate in peripheral nerves of diabetic patients and that the use of anti-AGE agents improves nerve conduction velocities and neuronal blood flow abnormalities. Aminoguanidine prevented diabetic nephropathy in diabetic animal models and was recently shown to do the same in one clinical trial on diabetic patients. Atherosclerosis is significantly accelerated in diabetic patients and is associated with greater risk of cardiovascular and cerebrovascular mortality. Animal and human studies have shown that AGEs play a significant role in the formation and progression of atherosclerotic lesions. Increased AGE accumulation in the diabetic vascular tissues has been associated with changes in endothelial cell, macrophage, and smooth muscle cell function.

In addition, AGEs can modify LDL cholesterol in such a way that it tends to become easily oxidized and deposited within vessel walls, causing streak formation and, in time, atheroma. AGE-crosslink formation results in arterial stiffening with loss of elasticity of large vessels. This arterial stiffness has recently been shown to be reversed by the administration of another anti-AGE class of compounds called AGE-breakers. [18] [19]

3. Diabetes Mellitus

Diabetes often referred to diabetes mellitus or simply diabetes. It describes as a group of chronic diseases in which the person has high blood glucose (blood sugar) that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces, or both. Insulin is a hormone that regulates blood sugar. Hyperglycaemia, or raised blood sugar, is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems, especially the nerves and blood vessels. Patients with high blood sugar will typically experience polyuria (frequent urination); they will become increasingly thirsty (polydipsia) and hungry (polyphagia).

There are three types of diabetes:

3.1 *Type 1 Diabetes*

Type 1 diabetes (previously known as insulin-dependent, juvenile or childhood-onset, or early-onset diabetes) is characterized by deficient insulin production and requires daily administration of insulin. The exact cause of type 1 diabetes is unknown and it is not preventable with current knowledge. In most people with type 1 diabetes, the body's own immune system – which normally fights harmful bacteria and viruses – mistakenly destroys the insulin-producing (islet) cells in the pancreas. Genetics may play a role in this process, and exposure to certain environmental factors, such as viruses, may trigger the disease.

Symptoms include excessive excretion of urine (polyuria), thirst (polydipsia), constant hunger, weight loss, vision changes and fatigue. These symptoms may occur suddenly. Various factors may contribute to type 1 diabetes, including genetics and exposure to certain viruses. Although type 1 diabetes usually appears during childhood or adolescence, it also can begin in adults. People usually develop type 1 diabetes before their 40th year, often in early adulthood or teenage years.

Despite active research, type 1 diabetes has no cure. But it can be managed. With proper treatment, people with type 1 diabetes can expect to live longer, healthier lives than did people with type 1 diabetes in the past. In type 1 diabetes, there's no insulin to let glucose into the cells, so sugar builds up in the bloodstream, where it can cause life-threatening complications. Patients with type 1 diabetes will need to take insulin injections for the rest of their life. They must also ensure proper blood-glucose levels by carrying out regular blood tests and following a special diet.

3.2 *Type 2 Diabetes*

Type 2 diabetes formerly called non-insulin-dependent or adult-onset diabetes. It is a chronic condition resulted from the body's ineffective use of insulin because body does not produce enough insulin for proper function, or the cells in the body do not react to insulin (insulin resistance). Thus, it affects the way to metabolize sugar (glucose), body's important source of fuel. Type 2 diabetes comprises 90% of people with diabetes around the world, and is largely the result of excess body weight and physical inactivity. With type 2 diabetes, the body either resists the effects of insulin or doesn't produce enough insulin to maintain a normal glucose level. By the way, type 2 diabetes symptoms often develop slowly. Symptoms may be similar to those of Type 1 diabetes, but are often less marked. However, type 2 diabetes is typically a progressive disease and the patient will probably end up have to take insulin, usually in tablet form.

Type 2 diabetes develops when the body becomes resistant to insulin or when the pancreas stops producing enough insulin. Exactly why this happens is unknown, although genetics and environmental factors, such as excess weight and inactivity, seem to be contributing factors. In type 2 diabetes, the process to keep glucose level in normal range doesn't work well. Instead of moving into cells, sugar builds up in the bloodstream. As blood sugar levels increase, the insulin-producing beta cells in the pancreas produce more insulin, but eventually these cells become impaired and can't make enough insulin to meet the body's demands. In the much less common type 1 diabetes, the immune system destroys the beta cells, leaving the body with little to no insulin.

More common in adults, type 2 diabetes increasingly affects children as childhood obesity increases. There's no cure for type 2 diabetes, but it can be able to manage the condition by eating well, exercising and maintaining a healthy weight. Overweight and obese people have a much higher risk of developing type 2 diabetes compared to those with a healthy body weight. People with a lot of visceral fat, also known as central obesity, belly fat, or abdominal obesity, are especially at risk. Being overweight/obese causes the body to release chemicals that can destabilize the body's cardiovascular and metabolic systems.

3.3 *Gestational Diabetes*

Gestational diabetes is hyperglycaemia with blood glucose values above normal but below those diagnostic of diabetes, occurring during pregnancy. Some women have very high levels of glucose in their blood, and their bodies are unable to produce enough insulin to transport all of the glucose into their cells, resulting in progressively rising levels of glucose.

Women with gestational diabetes are at an increased risk of complications during pregnancy and at delivery. They are also at increased risk of type 2 diabetes in the future.

The majority of gestational diabetes patients can control their diabetes with exercise and diet. Between 10-20% of gestational diabetes patients will need to take some kind of blood-glucose-controlling medications. Undiagnosed or uncontrolled gestational diabetes can raise the risk of complications during childbirth. The baby may be bigger than he/she should be.

The common consequences of diabetes are increasing the risk of heart disease and stroke. In a multinational study, 50% of people with diabetes die of cardiovascular disease (primarily heart disease and stroke). Neuropathy (nerve damage) in the feet increases the chance of foot ulcers, infection and eventual need for limb amputation when combined with reduced blood flow. Diabetic retinopathy is an important cause of blindness, and occurs as a result of long-term accumulated damage to the small blood vessels in the retina. One percent of global blindness can be attributed to diabetes. Diabetes is among the leading causes of kidney failure. Therefore, the overall risk of dying among people with diabetes is at least double the risk of their peers without diabetes.

3.4 *Complications linked to badly controlled diabetes*

(1) Eye complications – glaucoma, cataracts, diabetic retinopathy, and some others.

(2) Foot complications – neuropathy, ulcers, and sometimes gangrene which may require that the foot be amputated.

(3) Skin complications – people with diabetes are more susceptible to skin infections and skin disorders.

(4) Heart problems – such as ischemic heart disease, when the blood supply to the heart muscle is diminished.

(5) Hypertension – common in people with diabetes, which can raise the risk of kidney disease, eye problems, heart attack and stroke.

(6) Mental health – uncontrolled diabetes raises the risk of suffering from depression, anxiety and some other mental disorders.

(7) Hearing loss – diabetes patients have a higher risk of developing hearing problems.

(8) Gum disease – there is a much higher prevalence of gum disease among diabetes patients.

(9) Gastroparesis – the muscles of the stomach stop working properly.

(10) Ketoacidosis – a combination of ketosis and acidosis; accumulation of ketone bodies and acidity in the blood.

(11) Neuropathy – diabetic neuropathy is a type of nerve damage which can lead to several different problems.

(12) HHNS (Hyperosmolar Hyperglycemic Nonketotic Syndrome) – blood glucose levels shoot up too high, and there are no ketones present in the blood or urine. It is an emergency condition.

(13) Nephropathy – uncontrolled blood pressure can lead to kidney disease.

(14) Peripheral Arterial Disease (PAD) – symptoms may include pain in the leg, tingling and sometimes problems walking properly.

(15) Stroke – If blood pressure, cholesterol levels, and blood glucose levels are not controlled the risk of stroke increases significantly.

(16) Erectile dysfunction – male impotence.

(17) Infections – people with badly controlled diabetes are much more susceptible to infections

(18) Healing of wounds – cuts and lesions take much longer to heal [20] [21]

4. Laurel clock vine (*Thunbergia laurifolia*)

4.1 Description

Laurel clock vine (*T. laurifolia*) is commonly known as blue trumpet vine or Ran-jued in Thai language originated from southern China. It has been widely cultivated as a garden ornamental in tropical and sub-tropical regions for its attractive blue flowers. Flowers are attractive with pale purplish-blue petals and a yellow throat. Leaves are heart-shaped with a pointed tip and slightly serrated leaf margin. Therefore, the specific epithet "laurifolia" refers to its laurel-shaped leaves. The plant flowers almost continuously throughout the year with flowers opening early in the morning and aborting in the evening of the same day. The plant develops a very tuberous root system.

Laurel clock vine is the plant that used for antidote for a long time. The antidote activity of laurel clock vine is very broad which can neutralize pesticides, venom from plant and animals and other chemical poisons. Laurel clock vine also reduces the inflammation as well and more effective than mangosteen twice times and safer too. Most of antidote mechanism of laurel clock vine is anti-oxidative activity and it has active ingredients that can neutralize the toxins before they will react with cells.

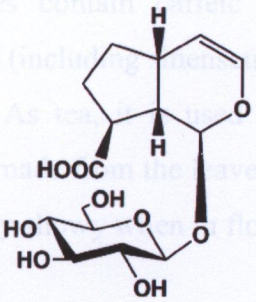
It is cultivated as an ornamental in gardens in tropical regions and in heated glasshouses in temperate regions. It is used medicinally in Thailand and Malaysia, although it is considered an invasive in other tropical regions.

Figure 4: Leaves and flowers of laurel clock vine or Ran-jued (*T. laurifolia*)

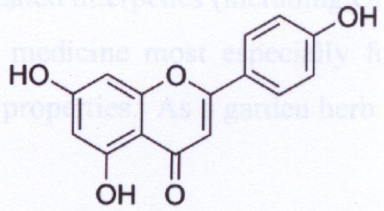
Laurel clock vine leaves are reported to have detoxifying effects, and in Thailand they are used as an antidote for poisons and in the treatment of drug addiction. Herbal teas and capsules containing laurel clock vine leaves are sold in Thailand. A Thai study published in 2012 suggested that laurel clock vine has antioxidant and anti-inflammatory properties. It concluded that it may be effective in treating inflammations caused by *Opisthorchis viverrinia* (Southeast Asian liver fluke), a parasite that attacks the liver and is endemic in northern areas of Thailand, Vietnam, Cambodia and Laos.

4.2 Active Compounds

The compounds isolated from the leaves included iridoid glucosides, grandifloric acid, glucopyranosides, and derivatives of apigenin. Other compounds found in leaves and flowers were delphinidin derivatives, and phenolic acids of chlorogenic, caffeic, gallic, and protocatechuic. Benefits of laurel clock vine are anti-oxidative activity, neutralize toxins and reduce the hazard from toxins, anti-diabetic activity, anti-inflammatory, hepatoprotective effect, treats drug addiction, and antimutagenicity. [22] [23]



(a)



(b)

Figure 5: Active compound found in laurel clock vine; Grandifloric acid (a) and Apigenin (b)

5. Cat's whisker (*Orthosiphon aristatus*)

5.1 Description

Cat's whisker (*O. aristatus*) is native to Southeast Asia and has spread to the East Indies, Indochina and Indonesia. It is also cultivated in Java and Sumatra. The plant owes its name to the four strikingly long stamens, which stick out, as long as a pen, looking like a cat's whiskers sticking out from the white to pale purple flowers lip. The flowers are in spike-like flowery whorls, which are expressed by the epithet: Lat "aristatus" (with ears). The genus name "Orthosiphon" comes from the Greek and means "upright tube".

Figure 6: Leaves and flowers of Cat's whisker (*O. aristatus*)

The perennial, herbaceous plants will grow up to 60 inches and has purple, four-sided stems that are coarsely toothed with pointed leaves, arranged in decussate. Leaves can be used fresh or dried as an infusion or decoction and are quite distinctive in flavor and aroma.

Cat's whisker leaves contain caffeic acid derivatives (including rosmarinic acid) and lipophilic flavonoids (including sinensetin) and oxygenated diterpenes (including Orthosiphol and Orthosiphon). As tea, it is used in traditional medicine most especially for kidney complaints, as a tea made from the leaves has diuretic properties. As a garden herb it is quite easy to grow and very showy when in flower.

5.2 Toxicity

Other than leaf, other parts of the plants are toxic and could not be used as medicine. According to a study done on Wistar rats on the chronic toxicity of water extract of Cat's whisker, high doses of the extract caused a reduction of serum sodium levels in all controlled groups. It also increases the alklaine phosphatase level and incidence of hydrocalyx in male rats. Therefore long term intake of Cat's whisker may cause undesirable side effects and is not advisable.

5.3 Medical Uses

Cat's whisker is known to use for treating of renal inflammation, kidney and urinary stone, treating of Dysuriam, reducing blood glucose level, improving diabetes, treating Arthritis, Rheumatism, and Gout, treating of Hypertension, and providing both antioxidant properties and anti-diabetic effects. [24] [25]

METHODOLOGY

1. Materials

Cat's whisker tea (*Orthosiphon aristatus*) and Laurel clock vine tea (*Thunbergia laurifolia*) was sponsored by Chinese tea shop®, Samui island, Thailand. All chemical reagents were food grade and available in laboratory.

2. Preparation of four model systems as control

Phosphate buffer were prepared by dissolving monosodium phosphate 3.173 g in 250 ml of distilled water and dissolving disodium phosphate 20.639 g in 250 ml of distilled water. Mix both solutions before adjusted volume to 1 L.

Fructose, glucose, lysine, and glycine were prepared at 0.5 M by using phosphate buffer (pH 7.39) as solvent. Four model systems were prepared by mixing the sugar solution and amino acid solution as fructose-lysine (Fru-Lys), fructose-glycine (Fru-Gly), glucose-lysine (Glu-Lys), and glucose glycine (Glu-Gly). The ratio of Fru-Lys and Glu-Lys were 1:1, while the ratio of Fru-Gly and Glu-Gly were 1:2.

To develop the glycation reaction, all model systems were incubated at 60°C for 7 hours.

3. Study effect of the concentration of Cat's Whisker tea and Laurel clock vine tea on the development of glycation in model system

3.1 Tea preparation

Cat's whisker tea and Laurel clock vine tea were weighing 0.5, 0.75, and 1 g and put in the tea bag before brewed with 100 mL of hot water (90°C) for 15 minutes.

3.2 Investigate the development of glycation in model systems at OD420 nm

Cat's whisker tea and Laurel clock vine tea were prepared at various concentrations. Tea solution was added into the model systems in the ratio 1:1 before heated at 60°C. Samples from all model systems were sampling every hour for 7 hours to measure the glycation development at OD420 nm. The results were analyzed with statistic to select the best condition to retard the glycation of each model system.

4. Investigate the effect of the best concentration of tea solution on the development of glycation using High Performance Liquid Chromatography (HPLC) method

4.2.1 ***Sample preparation for HPLC analysis***

Samples from the best condition and the control of all model systems were prepared before analyzed by HPLC. Samples were added 0.7 ml of 10% TCA before centrifuged at 10000 rpm for 15 minutes. The supernatant was adjusted to be neutral with 2N NaOH before subjected into HPLC.

4.2.2 ***Investigate the development of HMF***

The amount of HMF in the samples were analyzed by HPLC (Shimadzu HPLC HGE system) using inersil-ODS column (5 μm, 250 × 4.6 mm) at 40°C. Mobile phase was 5 % acetonitrile in 0.2 % phosphoric acid at the flow rate of 1 mL/min. The detector used was UV detector at 280 nm. Standard solution of 5-HMF were prepared at 0.1, 0.5, and 1.0 mg/L.

4.2.2 ***Investigate the sugar content***

The amount of glucose and fructose in the samples were analyzed by HPLC using inersil-NH_2 column (5 μm, 250 × 4.6 mm) at 40°C. Mobile phase was acetronile-water in the ratio of 3:1 at the flow rate of 1 mL/min. Detector used was refractive index (RI) detector. Standard sugar solutions were prepared at 2, 4, and 6 g/L.

RESULTS AND DISCUSSION

1. Study effect of the concentration of Cat's Whisker tea and Laurel clock vine tea on the development of glycation in model system

According to the medicinal properties, Cat's Whisker tea were reported for reducing blood glucose level and improving diabetes, while Laurel clock vine tea were known about its antioxidant and anti-inflammatory properties. Thus, Cat's Whisker tea and Laurel clock vine tea were used to study the ability to retard glycation development in sugar-amino acid model systems.

In the study, phosphate buffer (pH 7.39) was used as the solvent for all sugar and amino acid solutions. Four model systems were prepared by mixing the sugar solution and amino acid solution as glucose-Glycine (Glu-Gly), fructose-glycine (Fruc-Gly), glucose-lysine (Glu-Lys) and fructose-lysine (Fruc-Lys). The ratio of Fruc-Lys and Glu-Lys were 1:2 whereas the ratio of Fruc-Gly and Glu-Gly were 1:1 because lysine contains 2 amino groups while glycine contains only one amino group. Each model system was incubated at 60°C for 7 hours to develop glycation. This incubation condition was chosen from the previous study as it was the most appropriate condition to observe the change of glycation. . After every hour, the incubated solution was sampled to observe the development of glycation reaction at OD 420.

In figure 7, the results of glycation for all model systems were shown. Different models showed different rate of glycation. The highest rate of glycation reaction was of Glu-Lys model, following with Fruc-Lys, Fruc-Gly, and Glu-Gly, respectively. Those results implied that when the sugar was mixed with amino, glycation or browning occurred and lysine developed glycation faster than glycine. Because lysine contained two amino acid groups, while glycine contained only one amino acid group.

To study the effect of Cat's Whisker tea and Laurel clock vine tea on the development of glycation, both teas were prepared separately by steeping tea 0.5, 0.75, and 1.0 grams with 100 mL hot water (90°C) for 15 minutes. Tea solution was added into the model systems in the ratio 1:1 before heated at 60°C for 7 hours to develop glycation. After every hour, the incubated solution was sampled to observe the development of glycation reaction at OD 420. The results of this studied were shown in figure 8 to11.

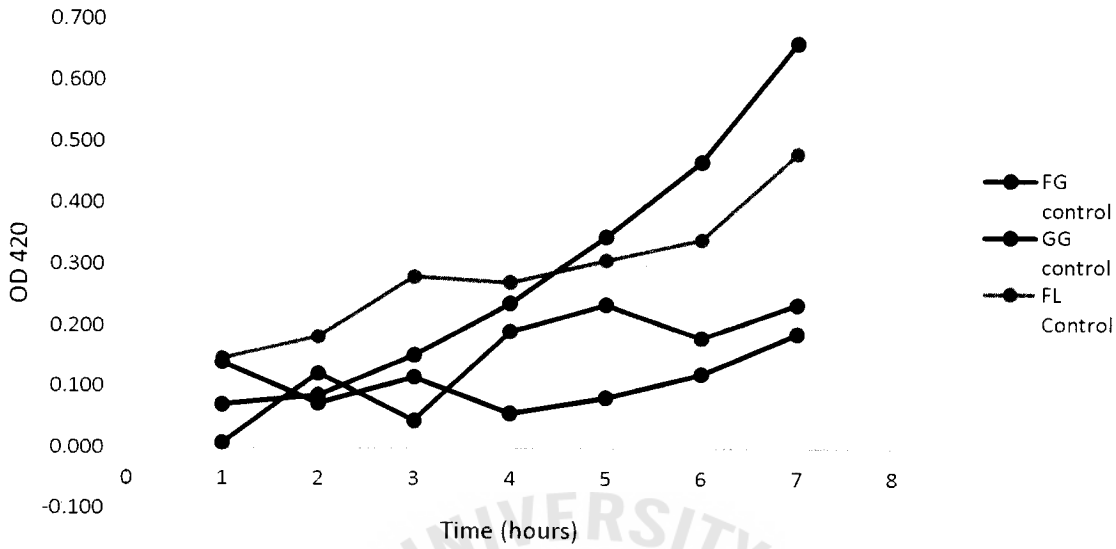


Figure 7: Development of glycation in all model systems heated at 60°C for 7 hours; Fruc-Gly , Glu-Gly , Fruc-Lys and Glu-Lys

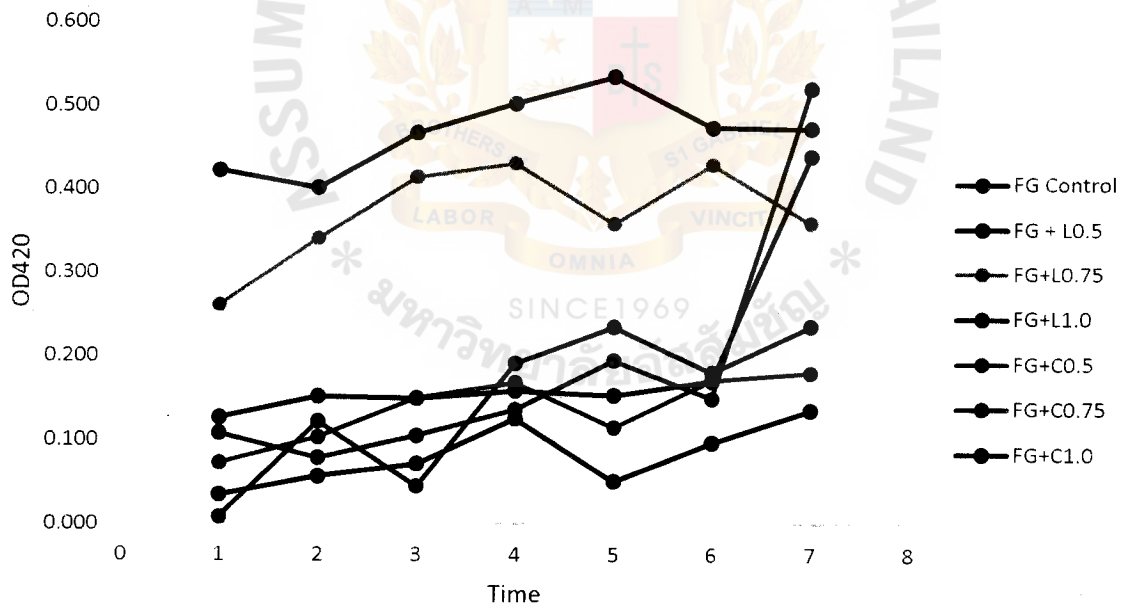


Figure 8: Development of glycation in Fruc-Gly models with various percentage of tea addition and heated at 60°C for 7 hours

In figure 8, the initial point of glycation developed in Fruc-Gly model with Laurel clock vine tea addition at 0.75% (green line) and 1.0% (purple line) were higher than control

(without tea addition). The reason resulted from the tea color which was pale brown color and this cause the higher initial point of the reaction than control and other models. For the development of glycation in Fruc-Gly models, the fastest development was found in Cat's Whisker tea at 0.75% addition, while the slowest rate of glycation was found in the addition of 1.0% Cat's Whisker tea and 0.5% Laurel clock vine tea. The slowest rate of glycation implied to the better antiglycation than others. Thus, both models with tea addition showed the lowest rates of glycation were selected for further analysis.

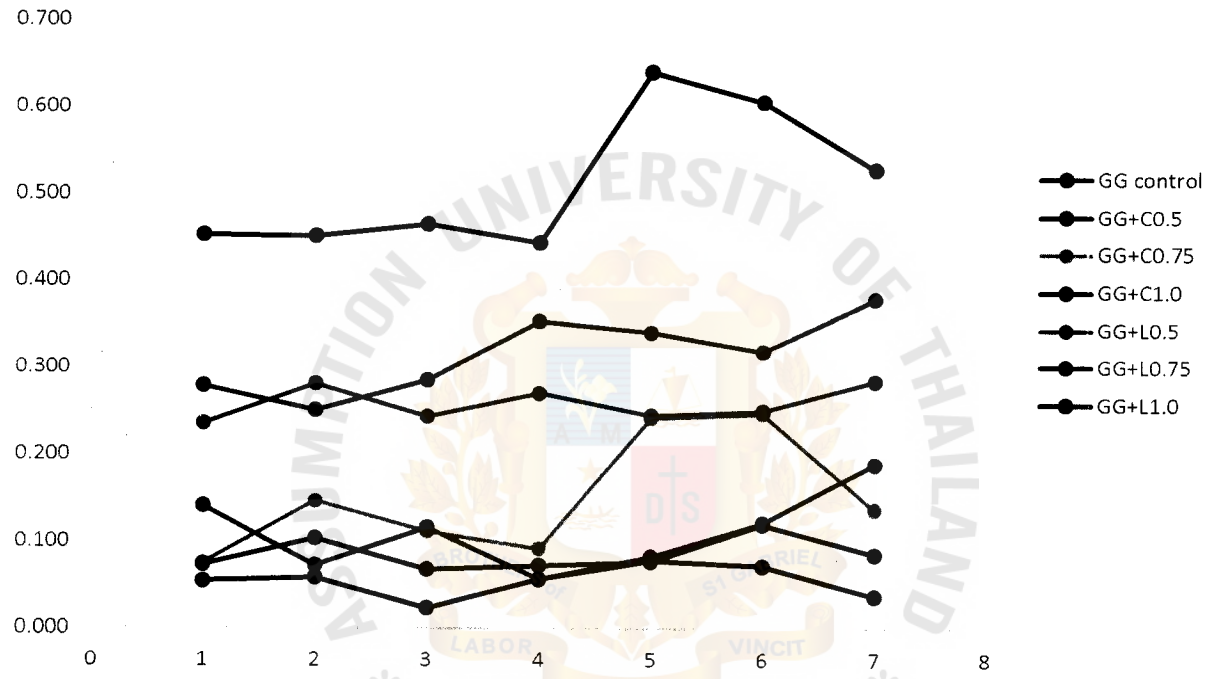


Figure 9: Development of glycation in Glu-Gly models with various percentage of tea addition and heated at 60°C for 7 hours

In figure 9, all models showed the relatively constant rate of glycation. The increasing of glycation slowed. The models with Laurel clock vine tea addition also showed the higher color intensity at the beginning than control. The higher tea concentration, the more color intensity.

When the Cat's Whisker tea was added into the model at 0.5 and 1.0%, the rate of browning reactions significantly decreased. This result implied that both the tea concentrations had the antiglycation properties. Thus, they can be able to retard the brown

color development. As the previous models, the best antiglycation of the model with tea addition were selected for further analysis according to its low rate of reaction.

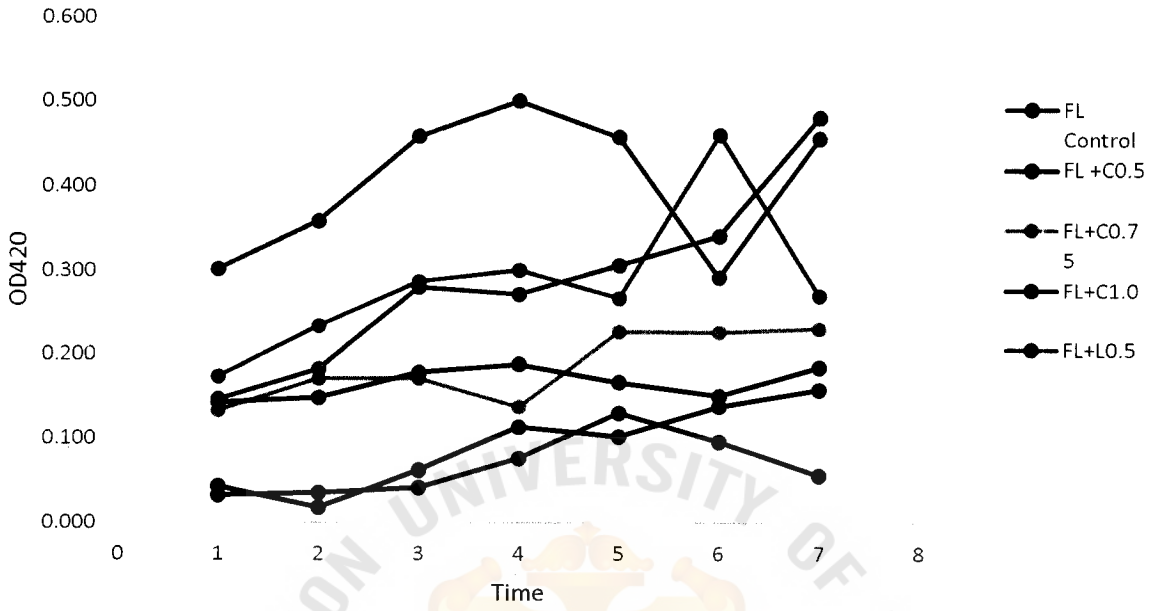


Figure 10: Development of glycation in Fruc-Lys models with various percentage of tea addition and heated at 60°C for 7 hours

Figure 10 showed the comparison of glycation reactions in Fruc-Lys models with or without tea addition. When compared with control (rate of glycation was 0.048), after the teas both Cat's Whisker tea and Laurel clock vine tea were added into the model system at various concentrations, the rate of glycation significantly decreased. This result implied that the teas had the antiglycation properties; therefore they can be able to retard the brown color development. Among the Cat's Whisker tea additions, even though 0.75% tea showed the higher initial color intensity than 0.5 and 1.0% tea, it showed the development of glycation slower than 0.5% tea addition and faster than 1.0% tea addition. Both 0.5 and 1.0% Cat's Whisker tea addition showed the same increasing rate of glycation within the first 5 hours of incubation. However, during the last 2 hours of incubation, the rate of glycation in Fruc-Lys models with 0.5% Cat's Whisker tea addition continued increased, while the rate of glycation in Fruc-Lys models with 1.0% Cat's Whisker tea addition decreased making the total rate of glycation of 1.0% Cat's Whisker tea lower than 0.5% Cat's Whisker tea addition.

For Fruc-Lys models with Laurel clock vine tea addition, even though the rate of glycation in Fruc-Lys models with 0.5 and 0,75% tea addition were lower than control, the glycation development during the first four hours of incubation was rapidly increased. Not only that, the initial color intensity was higher than control. Moreover, the fluctuation of color measurement during the last two hours of incubation showed the unpredictable of both models. Thus, for Fruc-Lys models with tea addition, 1.0% Cat's Whisker tea and 1.0 % Laurel clock vine tea were selected for further analysis.

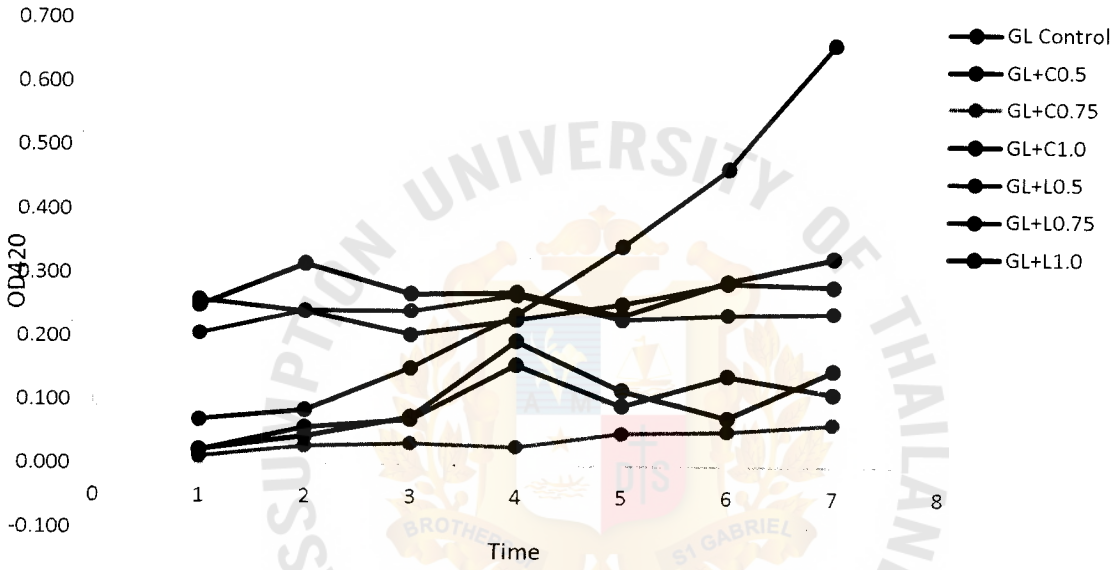


Figure 11: Development of glycation in Glu-Lys models with various percentage of tea addition and heated at 60°C for 7 hours

In figure 11, the development of glycation in Glu-Lys models without tea addition was sharply increased with the highest rate at 0.097. When tea was added, the rate of glycation was slowed or almost constant for all models. Cat's Whisker tea and Laurel clock vine tea addition show the different level of color initiation as Cat's Whisker tea addition showed the lower color intensity than Laurel clock vine tea addition. Among the different tea concentrations, Laurel clock vine tea addition showed no significantly differences of tea concentrations, while 0.75 % Cat's Whisker tea showed the lowest glycation development. Thus, 0.5% Laurel clock vine tea was selected for further analysis because the lower the Laurel clock vine tea used, the cheaper the price paid.

Therefore, from the development of glycation in four different model systems, the selected models with tea addition for further analysis were 1.0% Cat's Whisker tea and 0.5% Laurel clock vine tea for Fruc-Lys model, 0.5% Cat's Whisker tea for Glu-Gly model, 1.0% Cat's Whisker tea and 1.0% Laurel clock vine tea for Fruc-Lys model, and 0.75% Cat's Whisker tea and 0.5% Laurel clock vine tea for Glu-Lys model.

2. Investigate the effect of tea concentrations on the change of reducing sugar and HMF during the development of glycation

Reducing sugar is one of the important reactant of glycation. As the theory, the amount of reducing sugar will reduce during the glycation development implying the progress of the reaction. For HMF, it is an important intermediate of glycation occurring during the first and the second stage of glycation reaction. The accumulation of HMF implied the progress of glycation. When the amount of HMF reduced, it can be interpreted that the glycation was ran into the advance stage or the final stage which the highly final products of glycation were produced. Both reducing sugar content and amount of HMF are the indexes used to observe the progress of glycation development.

2.1 The change of reducing sugar during the development of glycation

Table 1: Change of fructose content in Fruc-Lys and Fruc-Gly models during heating at 60°C for 7 hours

Time (hrs)	Fruc-Lys			Fruc-Gly		
	Control (µg/mL)	**1.0%C*** (µg/mL)	**1.0%L**** (µg/mL)	**Control** (µg/mL)	**1.0%C*** (µg/mL)	**0.5%L**** (µg/mL)
1	1.309	0.081	0.413	0.635	0.515	0.576
2	1.299	0.130	0.361	0.622	0.630	0.526
3	1.059	0.131	0.513	0.334	0.888	0.567
4	0.957	0.041	0.492	0.448	0.887	0.571
5	0.946	0.053	0.478	0.420	0.541	0.512
6	0.932	0.085	0.507	0.456	0.905	0.460
7	1.056	ND***	0.520	0.438	0.757	0.412

Note: * *C is represented Cat's Whisker tea*
** *L is represented Laurel clock vine tea*
*** *ND is No Data*

Table 2: Change of glucose content in Glu-Lys and Glu-Gly models during heating at 60°C for 7 hours

Time (hrs)	Glu-Lys			Glu-Gly	
	Control (μg/mL)	**0.75%C*** (μg/mL)	**0.5%L**** (μg/mL)	**Control** (μg/mL)	**0.5%C*** (μg/mL)
1	0.682	0.422	0.365	0.578	0.294
2	0.867	0.411	0.388	0.638	0.170
3	0.764	0.429	0.484	0.680	0.194
4	0.557	0.388	0.454	0.831	0.162
5	0.590	0.499	0.434	0.695	0.205
6	1.034	0.554	0.336	0.644	0.264
7	1.091	0.452	0.249	0.327	0.194

Note: * *C is represented Cat's Whisker tea*
** *L is represented Laurel clock vine tea*

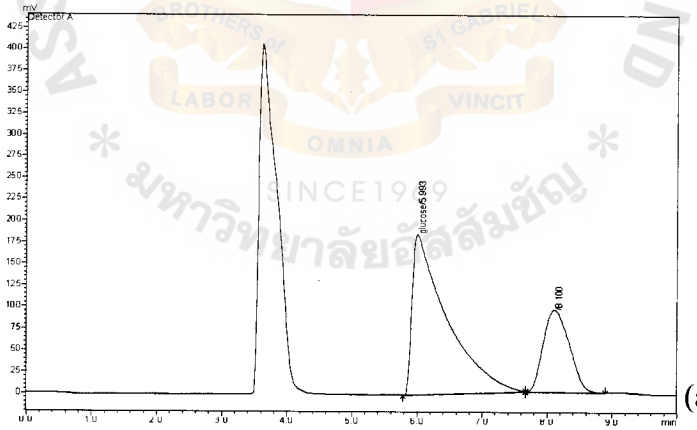
Table 1 and 2 showed the change of sugar content during glycation development at 60°C for 7 hours. The initial sugar content in the table were different because the different amount of sugar used in each model and the reaction started to observe the change of sugar after the glycation develop after 1 hour. It was not start at the beginning because it didn't show the development of glycation.

As the theory, the amount of sugar in the model implied the remaining free sugar that not reacted with amino acid. The lower the sugar content, the higher the glycation development. In Fruc-Lys model without tea addition, it showed the dramatically reduction of fructose. This result implied that fructose was used to react with lysine to develop glycation very fast. When compared with the brown color development, the results showed the reasonable trend of glycation. Fructose decreased, while brown color increased. While fructose in Fruc-Lys model (control) theoretically decreased, fructose in Fruc-Lys model with tea addition both 1.0% Cat's Whisker tea addition and 1.0% Laurel clock vine tea showed the constant of fructose level. The results explained the less fructose used to progress the glycation reaction.

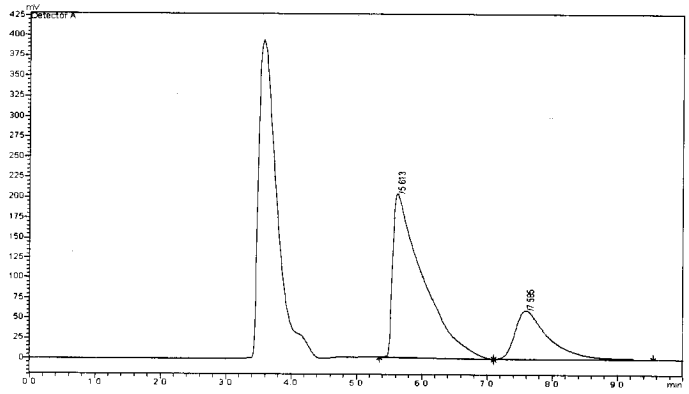
In Fruc-Gly model, fructose tended to decrease through the glycation development for both control and 0.1% Laurel clock vine tea addition, while 1.0% Cat's Whisker tea showed the increasing of fructose during the first three hours and then constant until 7th hour. The increasing of sugar in Fruc-Gly model with 1.0% Cat's Whisker tea addition showed the contrast with the hypothesis. The reason for this phenomenon was because of the sugar fragmentation which occurred during the advance stage of glycation. The fragment of sugar can be reformed to get new sugar molecules and caused the higher amount of sugar content in the model. By the way, the decreasing of fructose was not sharply decreased. The results

showed the same trend with brown color development because brown color also not increased rapidly. Thus, for Fruc-Gly model, the glycation occurred slower than Fruc-Lys model.

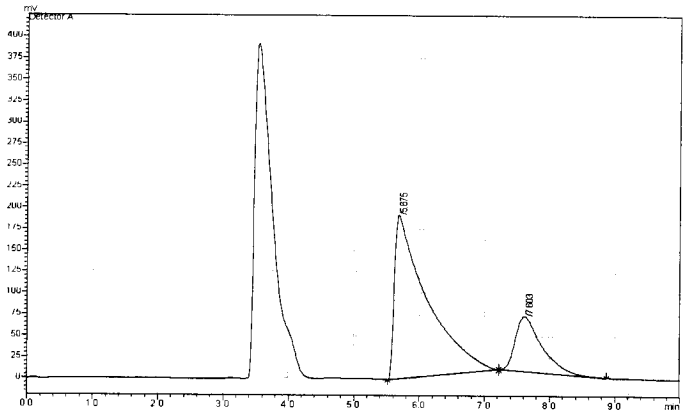
In Table 2, sugar content in Glu-Lys model without tea addition showed highly fluctuation of the sugar content because it shortly increased, then decreased, and finally raised up in the last 2 hours of incubation period. Glu-Lys model with 0.75% Cat's Whisker tea showed slowly progress of glycation because the rate of decreasing of sugar was not high. Sugar content in this model slowly increased but it tended to decreased in the last hour of incubation period. If the incubation was extended, the decreasing will be seeing more clearly. While the sugar content in Glu-Lys model with 0.75% Cat's Whisker tea tended to decreased, it tended to decrease for Glu-Lys model with 0.5% Laurel clock vine tea addition. The results showed that the rate of glycation for 0.5% Laurel clock vine tea addition was lower than 0.75% Cat's Whisker tea addition. While the glycation for 0.75% Cat's Whisker tea addition was progress to the further stage, the glycation for 0.5% Laurel clock vine tea addition was in the first stage that sugar or one of reactants starting to react and changed because the decreasing of sugar. The low decreasing of sugar implied the slow glycation development.



(a)



(b)



(c)

Figure 12: Example of sugar chromatogram of Fruc-Gly model; control (a), with 1.0% Cat's Whisker tea addition (b), and 0.5% Laurel clock vine tea addition (c)

For Glu-Gly model, control showed the increasing of sugar during the first 3four hours of incubation, and then decreased, while model with 0.5% Cat's Whisker tea addition showed the constant level of sugar. The results meant tea additions can retard glycation reaction because of less change of sugar and brown color development.

3. Investigate the effect of tea concentrations on the change of HMF content during the development of glycation

Table 3 and 4 showed the change of HMF content during glycation development at 60°C for 7 hours. HMF is the important intermediate occurred by dehydration of hexose sugars and it always used as an index to observe the development of glycation or, in the other hand, glycation reaction. As it is an intermediate, the increasing of HMF implied the accumulation of the brown pigment. However, it does not mean that it will accumulate through the end of the reaction. It can be changed to other products or other intermediates and caused the decreasing of HMF in the system.

Table 3: HMF content in Fruc-Lys and Fruc-Gly models during heating at 60°C for 7 hours

Time (hrs)	Fruc-Lys			Fruc-Gly		
	Control (μg/mL)	1.0%C* (μg/mL)	1.0%L** (μg/mL)	Control (μg/mL)	1.0%C* (μg/mL)	0.5%L** (μg/mL)
1	11.704	16.915	11.715	9.095	26.121	4.760
2	11.706	16.954	11.716	9.103	34.645	4.770
3	11.703	16.957	11.699	9.005	34.651	4.893
4	11.726	16.923	11.702	9.085	34.623	4.854
5	11.708	16.837	11.703	9.088	34.593	4.871
6	11.707	16.890	11.705	9.100	34.619	4.762
7	11.711	16.851	11.701	9.077	34.610	4.762

Note: * *C is represented Cat's Whisker tea*
** *L is represented Laurel clock vine tea*

Table 4: HMF content in Glu-Lys and Glu-Gly models during heating at 60°C for 7 hours

Time (hrs)	Glu-Lys			Glu-Gly	
	Control (μg/mL)	0.75%C* (μg/mL)	0.5%L** (μg/mL)	Control (μg/mL)	0.5%C* (μg/mL)
1	23.576	18.937	ND***	8.992	8.995
2	23.575	18.939	23.592	9.000	8.991
3	23.619	18.975	23.626	9.002	8.994
4	23.595	18.944	23.719	8.998	8.995
5	23.608	18.935	23.611	9.001	9.004
6	23.603	18.936	23.558	8.995	9.007
7	23.623	18.938	23.752	8.994	8.999

Note: * *C is represented Cat's Whisker tea*
** *L is represented Laurel clock vine tea*
*** *ND is No Data*

As shown in the table 3, the HMF in all Fruc-Lys and Fruc-Gly models showed the constant value with the different initial content. For Fruc-Lys models, the amount of HMF in 1.0% Cat's Whisker tea addition showed the higher HMF than control and 1.0% Laurel clock vine tea addition, that were approximately 16.9 μg/mL for 1.0% Cat's Whisker tea addition, and approximately 11.7 μg/mL for both control and 1.0% Laurel clock vine tea addition. The high HMF content in 1.0% Cat's Whisker tea addition with the constant level during the incubation can be explained that the glycation rapidly occurred in the initial stage but it didn't change to other intermediates products. Thus, the HMF content, brown color development, and the change of sugar implied that the addition of 1.0% Cat's Whisker tea can retard the glycation better than 1.0% Laurel clock vine tea.

For Fruc-Gly models, 1.0% Cat's Whisker tea addition rapidly increased during the first two hours of incubation, and then it was constant. The high HMF content were approximately 34.6 μg/mL followed by control (approximately 9.0 μg/mL) and 0.5% Laurel clock vine tea addition (approximately 4.8 μg/mL). For the control, HMF was slightly

increased because HMF was not being used up in the reaction. When 0.5% Laurel clock vine tea is added, the amount of HMF was low because HMF was changed to other compounds in the chain of glycation to develop the brown color. This result showed that when 0.5% Laurel clock vine tea is added into Fruc-Gly models, it has the capacity to retard the progress of glycation and therefore the browning reaction rate slows down.

In Table 4, Glu-Lys model with or without tea addition showed the constant level of HMF during incubation. The amount of HMF in control, 0.75% Cat's Whisker tea addition, and 0.5% Laurel clock vine tea addition was approximately 23.6, 18.9, and 23.5 µg/mL, respectively. The lower amount of HMF in Glu-Lys model with 0.75% Cat's Whisker tea addition illustrated that with 0.75% Cat's Whisker tea developed the glycation less than control and 0.5% Laurel clock vine tea addition.

For Glu-Gly model, at the beginning of incubation, amount of HMF in both control and tea addition was equal. However, HMF in control model was increased in the first two hours of incubation, and then decreased, while HMF in Glu-Gly model with 0.5% Cat's Whisker tea addition increased for 6 hours, and then decreased. When compared with the brown color development, control model showed a higher progress than 0.5% Cat's Whisker tea addition as HMF was not completely converted to give the color compounds.

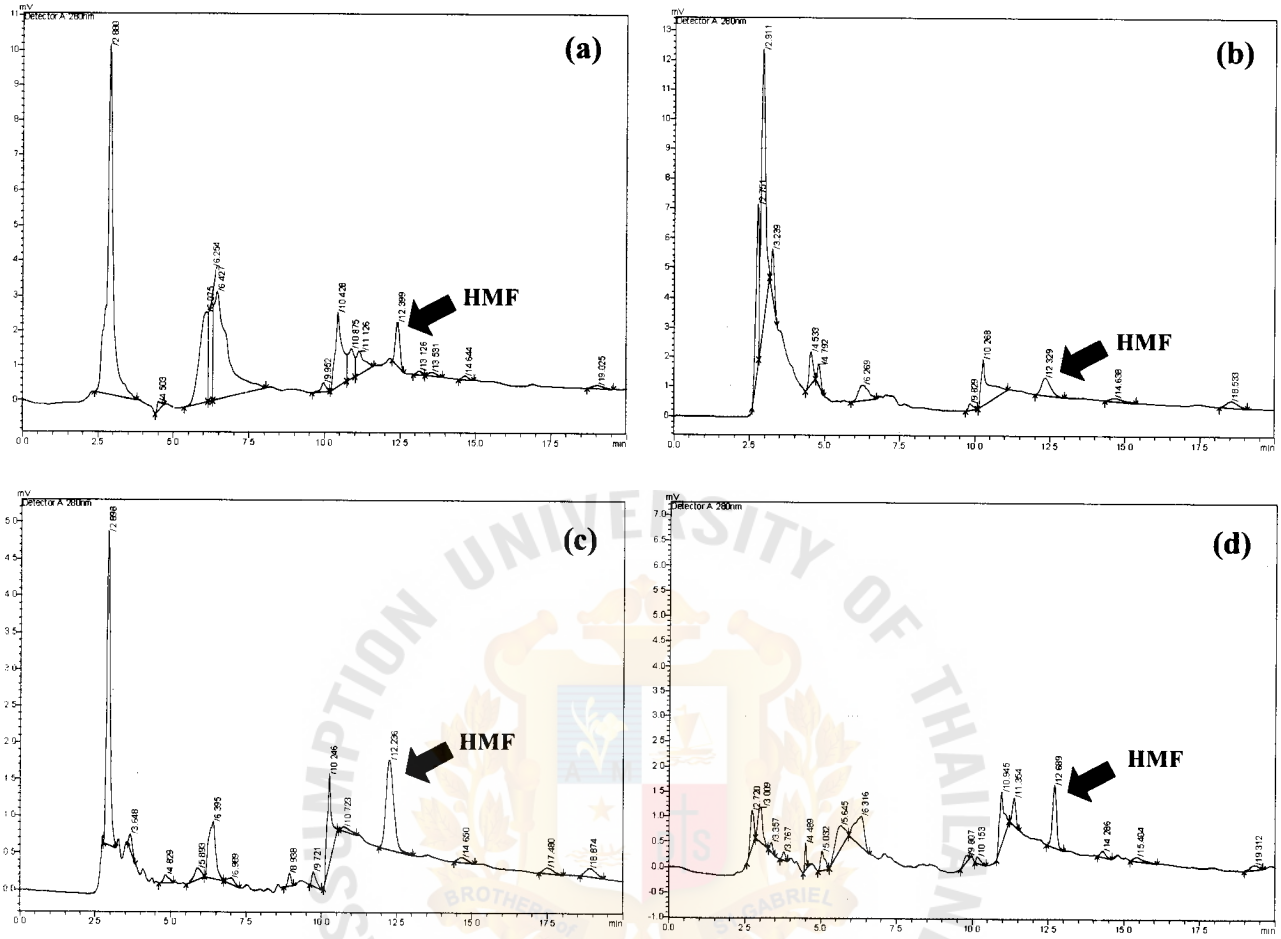


Figure 13: Example of HMF chromatogram of (a) Fruc-Lys model; (b) Fruc-Gly model; (c) Glu-Gly model with 1.0% Cat's Whisker tea addition; and (d) Glu-Lys with 0.75% Cat's Whisker tea addition

CONCLUSION

1. The highest rate of glycation was Glucose-Lysine model followed by Fructose-Lysine, Fructose-Glycine, and Glucose-Glycine, respectively.

2. The best tea and concentration used to retard the glycation in each model system was;
   2.1 Cat's Whisker tea 0.75% in Glucose-Lysine model
   2.2 Cat's Whisker tea 1.0% in Fructose-Lysine model
   2.3 Laurel clock vine tea 0.5% in Fructose-Glycine model
   2.4 Cat's Whisker tea 0.5% in Glucose-Glycine model

REFERENCES

1. http://www.wisegeek.org/what-is-glycation.htm#didyouknowout

2. http://en.wikipedia.org/wiki/Glycation

3. Odjakova Mariela, Popova Eva et al. 2012. " Plant Derived Agents with Anti Glycation Activity" edited by Stefana Petrescu, ISBN 978-953-51-0771-2, pp. 1-2

4. Nagai, Ryoji, et al. 2012. "Advanced glycation end products and their receptors as risk factors for aging." J Anti Aging Med. 9 (4): 108-113.

5. Uribarri, Jaime, et al. 2010. "Advanced glycation end products in foods and a practical guide to their reduction in the diet." J Am Diet Assoc. 110(6): 911-916.

6. Delgado Cristina, Seiquer Isabel et al. 2010. "Development of the Maillard reaction in foods cooked by different techniques. Intake of Maillard-derived compounds" Food Chemistry 122, pp. 145-146

7. U. Jaime, W. Sandra et al " Advanced Glycation End Products In Food And A Practical Guide To Their Reduction In The Diet", J Am Diet Assoc. 2010 Jun; 110(6): 911–16.e12.

8. Fabíola Cristina de Oliveira1,2, Jane Sélia dos Reis Coimbra et al "Food Protein-Polysaccharides Conjugates Obtained via the Maillard Reaction: A Review",2014

9. Trumbo P, Schlicker S, Yates AA, Poos M; Food and Nutrition Board of the Institute of Medicine, The National Academies. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids.J Am Diet Assoc

10. http://en.wikipedia.org/wiki/Glycine

11. http://www.webmd.com/vitamins-supplements/ingredientmono-1072-glycine.aspx?activeingredientid=1072&activeingredientname=glycine

12. David A. Bender, "A Dictionary of Food and Nutrition", Oxford University Press 2009

13. Steven D. Ehrlich, NMD, Solutions Acupuncture, a private practice specializing in complementary and alternative medicine, Phoenix, AZ. Review provided by VeriMed Healthcare Network, 2011

14. http://en.wikipedia.org/wiki/Glucose

15. Khimia Uglevodov, Moscow, 1967. The Great Soviet Encyclopedia, 3rd Edition (1970-1979)

16. http://www.scienceofcooking.com/glucose.htm

17. http://en.wikipedia.org/wiki/Fructose

18. Melpomeni Peppa,MD; Jaime Uribarri,MD; et al "Glucose, Advanced Glycation End Products, and Diabetes Complications: What Is New and What Works", Clinical Diabetes Vol 21, Number 4, 2003

19. Dr. Ronald Klatz, Dr. Robert Goldman, "Anti-Aging Therapeutics Vol XIII", American Academy of Anti-Aging Medicine (A4M),2010

20. http://www.webmd.com/diabetes/

21. http://www.who.int/mediacentre/factsheets/fs312/en/

22. http://www.kew.org/science-conservation/plants-fungi/thunbergia-laurifolia-laurel-clock-vine

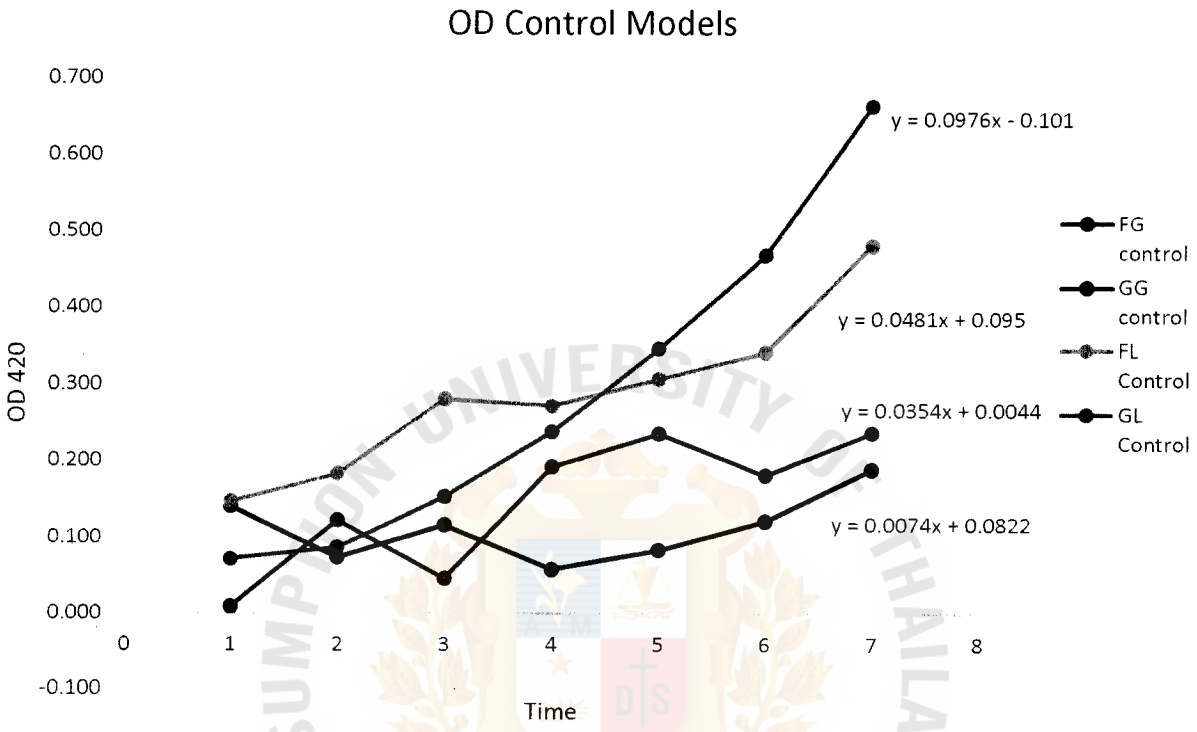
23. http://www.avaplant.com/products/raw-material-herbal-products/laurel-clock-vine-thunbergia-laurifolia/

24. http://www.medicalhealthguide.com/articles/orthosiphon.htm

25. http://www.webmd.com/vitamins-supplements/ingredientmono-707-java%20tea.aspx?activeingredientid=707&activeingredientname=java%20tea

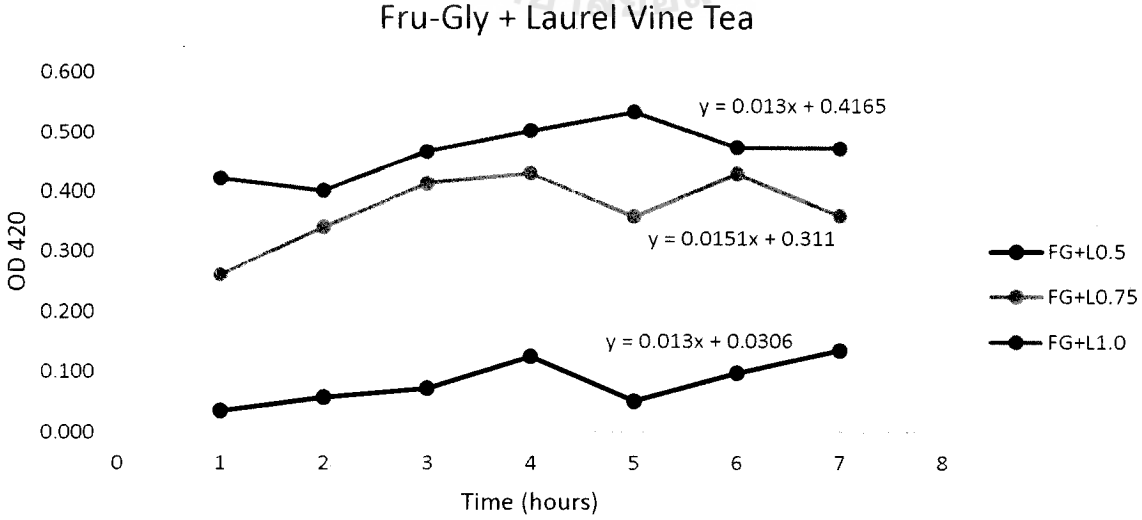
APPENDIX

1. Control models : Sugar and amino acids browning reactions



Sugar and amino acids browning reactions FG: Fructose-Glycine GG: Glucose-Glycine FL: Fructose-Lysine FG: Fructose-Glycine

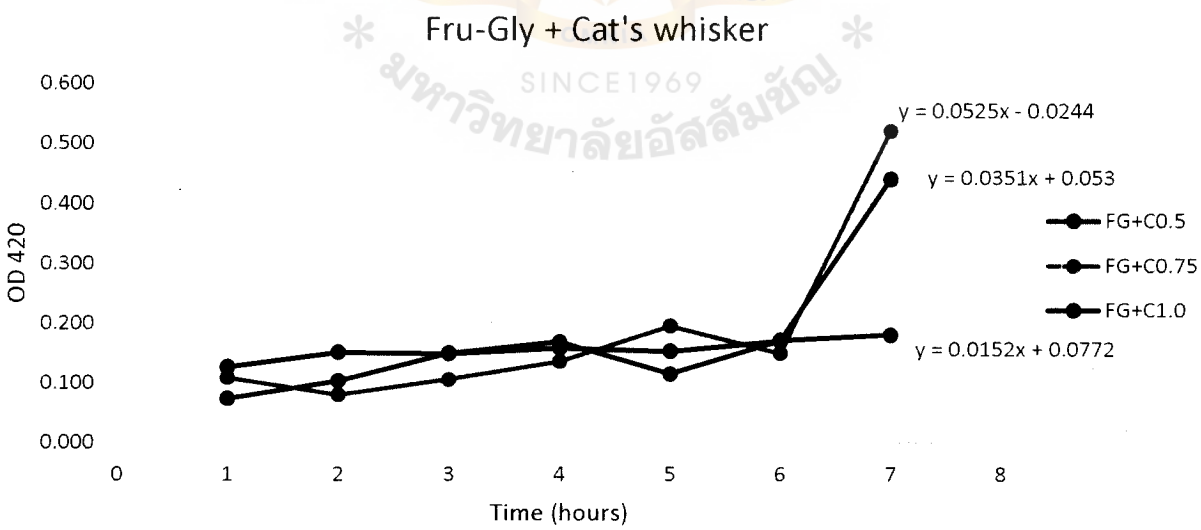
Fructose-Glycine Control					**Glucose - Glycine Control**				
Time (Hours)				Average	Time(Hours)				
1	0.022	0.051	-0.047	**0.009**	1	0.171	0.2	0.052	**0.141**
2	0.17	0.048	0.149	**0.122**	2	0.084	0.127	0.009	**0.073**
3	0.088	0.016	0.033	**0.046**	3	0.13	0.052	0.168	**0.117**
4	0.202	0.168	0.207	**0.192**	4	0.04	0.05	0.083	**0.058**
5	0.178	0.321	0.208	**0.236**	5	0.086	0.075	0.089	**0.083**
6	0.144	0.203	0.197	**0.181**	6	0.119	0.138	0.108	**0.122**
7	0.271	0.252	0.187	**0.237**	7	0.151	0.189	0.227	**0.189**
Fructose-Lysine Control					**Glucose-Lysine Control**				
Time (Hours)					Time(Hours)				
1	0.115	0.146	0.179	**0.147**	1	0.075	0.035	0.103	**0.071**
2	0.224	0.128	0.197	**0.183**	2	0.1	0.059	0.1	**0.086**
3	0.254	0.366	0.222	**0.281**	3	0.158	0.155	0.147	**0.153**
4	0.219	0.321	0.276	**0.272**	4	0.252	0.213	0.25	**0.238**
5	0.326	0.27	0.325	**0.307**	5	0.404	0.286	0.348	**0.346**
6	0.321	0.329	0.374	**0.341**	6	0.53	0.489	0.386	**0.468**
7	0.493	0.496	0.455	**0.481**	7	0.673	0.714	0.603	**0.663**



Fru-Gly Laurel Clock Vine Conc. 0.5				
Time				Average
1	0.034	0.02	0.052	0.035
2	0.075	0.05	0.048	0.058
3	0.134	0.064	0.019	0.072
4	0.264	0.01	0.104	0.126
5	0.093	0.034	0.028	0.052
6	0.068	0.171	0.055	0.098
7	0.045	0.119	0.245	0.136

Fru-Gly Laurel Clock Vine Conc 0.75				
Time				Average
1	0.254	0.274	0.255	0.261
2	0.412	0.281	0.331	0.341
3	0.435	0.438	0.371	0.415
4	0.464	0.419	0.412	0.432
5	0.337	0.301	0.441	0.360
6	0.366	0.51	0.415	0.430
7	0.37	0.379	0.333	0.361

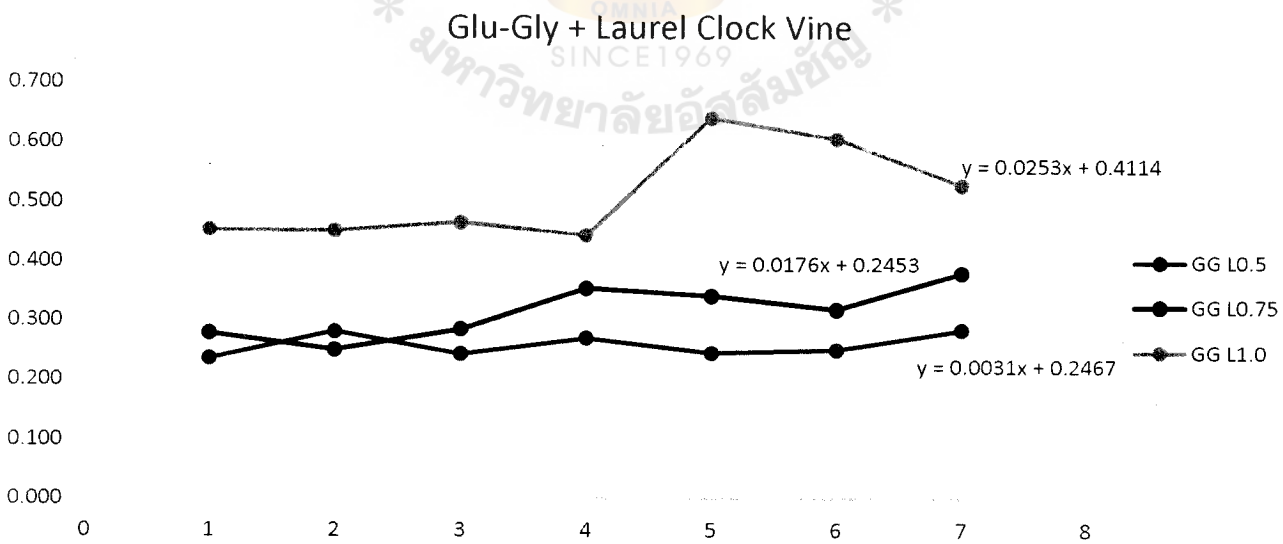
Fru-Gly Laurel Clock Vine Conc 1				
Time				
1	0.5	0.387	0.382	0.423
2	0.455	0.268	0.483	0.402
3	0.4459	0.513	0.446	0.468
4	0.449	0.527	0.534	0.503
5	0.504	0.596	0.505	0.535
6	0.505	0.426	0.494	0.475
7	0.448	0.5	0.473	0.474



Fru-Gly Cat's Whisker Conc 0.5				
Time	OD Values (420nm)			Average
1	0.038	0.102	0.08	0.073
2	0.017	0.155	0.139	0.104
3	0.125	0.218	0.109	0.151
4	0.147	0.138	0.223	0.169
5	0.146	0.138	0.063	0.116
6	0.167	0.185	0.165	0.172
7	0.214	0.151	0.178	0.181

Fru-Gly Cat's Whisker Conc 0.75				
Time	OD Values (420nm)			Average
1	0.136	0.126	0.064	0.109
2	0.05	0.1	0.089	0.080
3	0.134	0.117	0.067	0.106
4	0.14	0.164	0.106	0.137
5	0.159	0.179	0.249	0.196
6	0.058	0.236	0.157	0.150
7	0.477	0.334	0.753	0.521

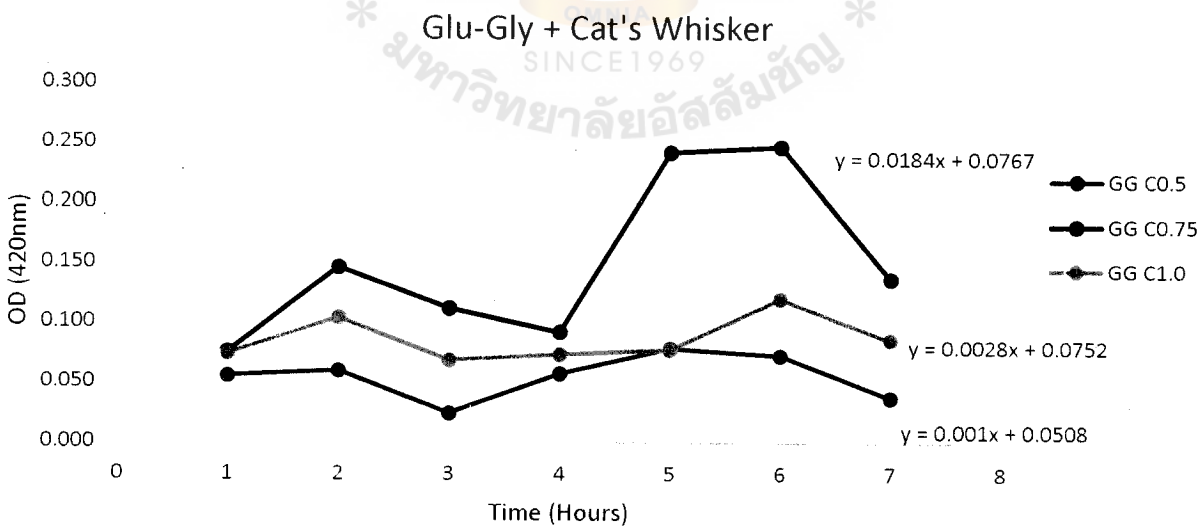
Fru-Gly Cat's Whisker Conc 1.0				
Time	OD Values (420nm)			Average
1	0.075	0.094	0.212	0.127
2	0.146	0.092	0.218	0.152
3	0.163	0.141	0.145	0.150
4	0.16	0.179	0.139	0.159
5	0.198	0.158	0.106	0.154
6	0.218	0.118	0.177	0.171
7	0.442	0.411	0.468	0.440



Glu-Gly Laurel Clock Vine Conc 0.5				
Time	OD Values (420nm)			Average
1	0.198	0.249	0.261	0.236
2	0.205	0.299	0.341	0.282
3	0.289	0.2	0.242	0.244
4	0.286	0.273	0.253	0.271
5	0.197	0.285	0.254	0.245
6	0.199	0.261	0.292	0.251
7	0.252	0.308	0.294	0.285

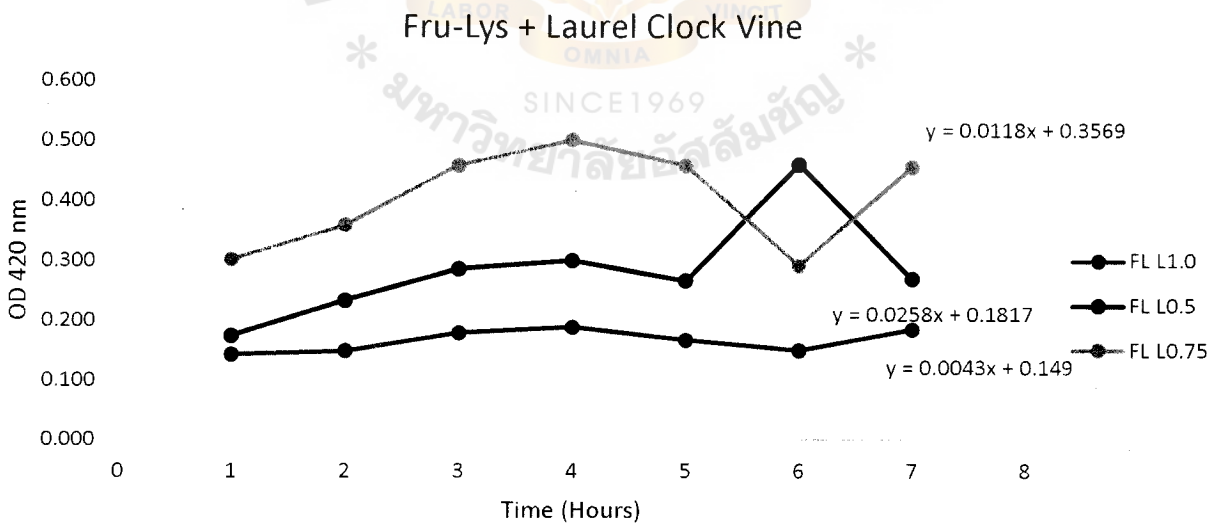
Glu-Gly Laurel Clock Vine Conc 0.75				
Time	OD Values (4220nm)			Average
1	0.315	0.209	0.314	0.279
2	0.299	0.158	0.296	0.251
3	0.264	0.264	0.329	0.286
4	0.292	0.455	0.315	0.354
5	0.339	0.329	0.356	0.341
6	0.294	0.312	0.35	0.319
7	0.348	0.391	0.401	0.380

Glu-Gly Laurel Clock Vine Conc 0.5				
Time	OD Values (420nm)			Average
1	0.402	0.468	0.489	0.453
2	0.366	0.368	0.621	0.452
3	0.497	0.452	0.447	0.465
4	0.46	0.457	0.415	0.444
5	0.521	0.541	0.859	0.640
6	0.45	0.497	0.871	0.606
7	0.585	0.454	0.545	0.528



Glu-Gly Cat's Whisker Conc 0.5					Glu-Gly Cat's Whisker Conc 0.75				
Time	OD Values (420nm)			Average	Time	OD Values (420nm)			Average
1	0.082	0.065	0.018	0.055	1	0.126	0.055	0.046	0.076
2	0.038	0.037	0.103	0.059	2	0.168	0.129	0.142	0.146
3	0.034	0.021	0.017	0.024	3	0.107	0.081	0.148	0.112
4	0.089	0.058	0.025	0.057	4	0.062	0.055	0.16	0.092
5	0.075	0.078	0.083	0.079	5	0.209	0.212	0.306	0.242
6	0.008	0.107	0.103	0.073	6	0.2	0.318	0.224	0.247
7	0.023	0.064	0.026	0.038	7	0.178	0.114	0.119	0.137

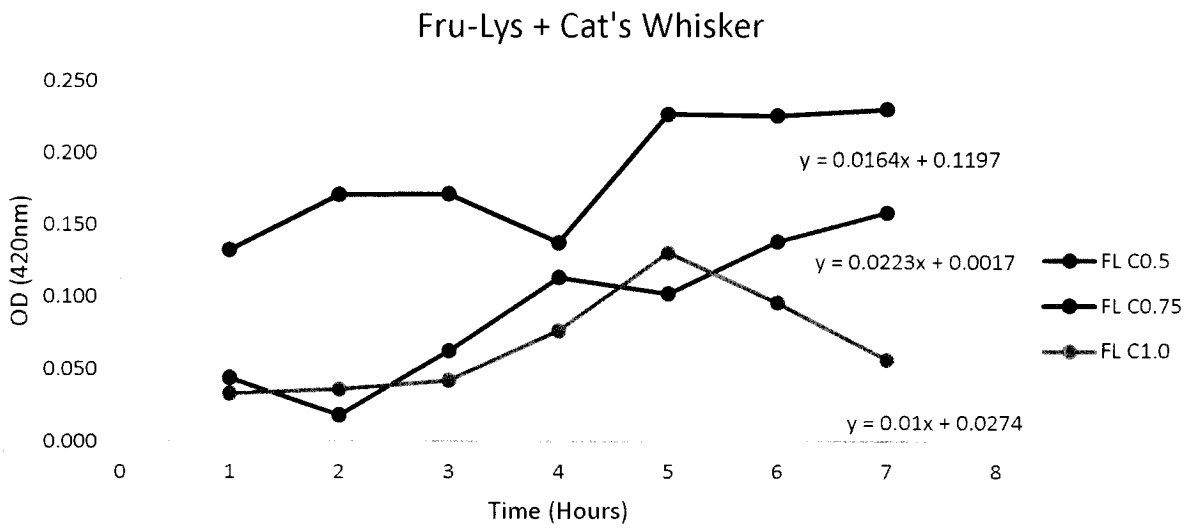
Glu-Gly Cat's Whisker Conc 1				
Time	OD Values (420nm)			Average
1	0.096	0.074	0.052	0.074
2	0.119	0.16	0.033	0.104
3	0.069	0.016	0.121	0.069
4	0.131	0.047	0.042	0.073
5	0.007	0.089	0.137	0.078
6	0.094	0.232	0.034	0.120
7	0.06	0.156	0.042	0.086



Fru-Lys Laurel Clock Vine Conc 0.5				
Time	OD Values (420nm)			Average
1	0.22	0.117	0.185	0.174
2	0.197	0.272	0.233	0.234
3	0.284	0.315	0.263	0.287
4	0.386	0.23	0.287	0.301
5	0.254	0.228	0.321	0.268
6	0.456	0.47	0.456	0.461
7	0.21	0.32	0.282	0.271

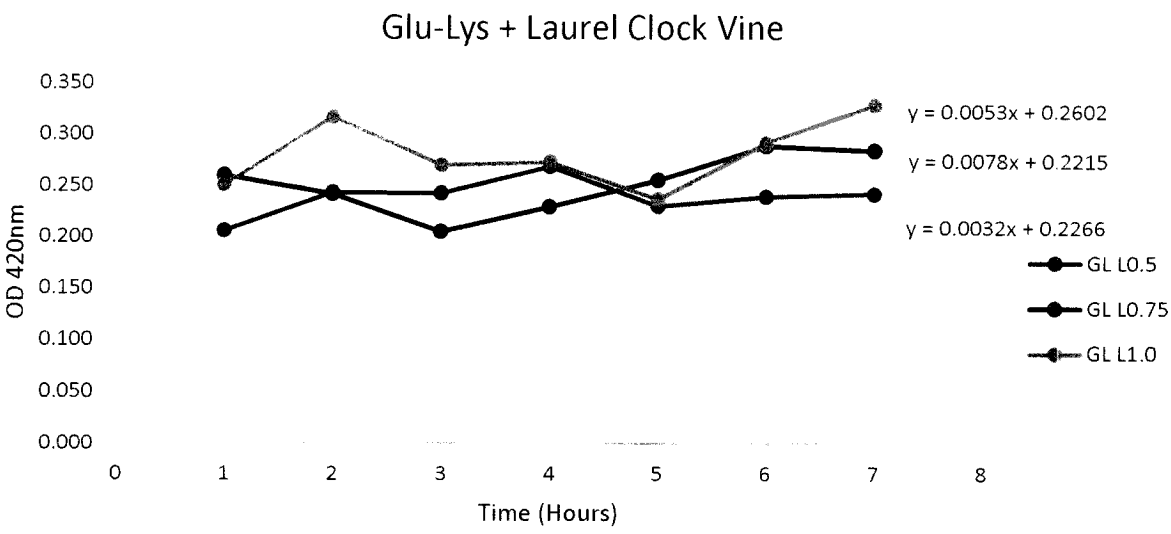
Fru-Lys Laurel Clock Vine Conc 0.75				
Time	OD Values (420nm)			Average
1	0.355	0.265	0.287	0.302
2	0.388	0.313	0.375	0.359
3	0.483	0.456	0.438	0.459
4	0.498	0.464	0.541	0.501
5	0.404	0.422	0.549	0.458
6	0.298	0.329	0.251	0.293
7	0.492	0.395	0.483	0.457

Fru-Lys Laurel Clock Vine Conc 1.0				
Time	OD Values (420nm)			Average
1	0.163	0.11	0.156	0.143
2	0.145	0.132	0.169	0.149
3	0.247	0.16	0.13	0.179
4	0.203	0.14	0.223	0.189
5	0.133	0.284	0.085	0.167
6	0.141	0.117	0.194	0.151
7	0.221	0.164	0.171	0.185



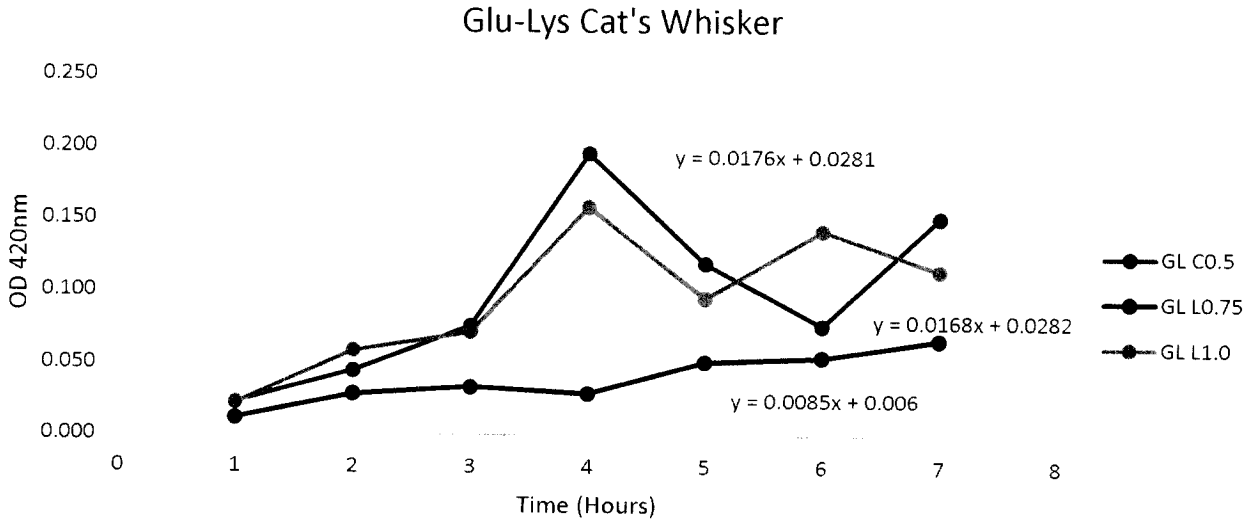
Fru-Lys Cats Whisker Conc 0.5					Fru-Lys Cats Whisker Conc 0.75				
Time	OD Values (420nm)			Average	Time	OD Values (420nm)			Average
1	0.069	0.014	0.048	0.044	1	0.14	0.132	0.126	0.133
2	0.021	0.019	0.015	0.018	2	0.189	0.134	0.191	0.171
3	0.087	0.025	0.075	0.062	3	0.179	0.103	0.233	0.172
4	0.101	0.107	0.132	0.113	4	0.19	0.098	0.125	0.138
5	0.044	0.11	0.153	0.102	5	0.244	0.268	0.17	0.227
6	0.126	0.051	0.238	0.138	6	0.23	0.219	0.23	0.226
7	0.123	0.182	0.171	0.159	7	0.255	0.176	0.261	0.231

Fru-Lys Cats Whisker Conc 1.0				
Time	OD Values (420nm)			Average
1	0.074	0.036	0.03	0.033
2	0.043	0.031	0.041	0.036
3	0.057	0.052	0.032	0.042
4	0.176	0.072	0.081	0.077
5	0.196	0.136	0.125	0.131
6	0.051	0.118	0.074	0.096
7	0.133	0.059	0.054	0.057



Glu-Lys Laurel Clock Vine Conc 0.5					Glu-Lys Laurel Clock Vine Conc 0.75				
Time	OD Values (420nm)			Average	Time	OD Values (420nm)			Average
1	0.226	0.218	0.175	0.206	1	0.26	0.269	0.252	0.260
2	0.251	0.285	0.195	0.244	2	0.27	0.197	0.26	0.242
3	0.16	0.302	0.267	0.243	3	0.244	0.2	0.173	0.206
4	0.233	0.274	0.3	0.269	4	0.192	0.29	0.208	0.230
5	0.265	0.254	0.173	0.231	5	0.263	0.23	0.276	0.256
6	0.268	0.248	0.203	0.240	6	0.282	0.287	0.299	0.289
7	0.263	0.226	0.239	0.243	7	0.26	0.299	0.296	0.285

Glu-Lys Laurel Clock Vine Conc 1.0				
Time	OD Values (420nm)			Average
1	0.288	0.257	0.209	0.251
2	0.318	0.241	0.392	0.317
3	0.22	0.312	0.28	0.271
4	0.225	0.35	0.246	0.274
5	0.207	0.253	0.252	0.237
6	0.3	0.331	0.245	0.292
7	0.424	0.332	0.231	0.329

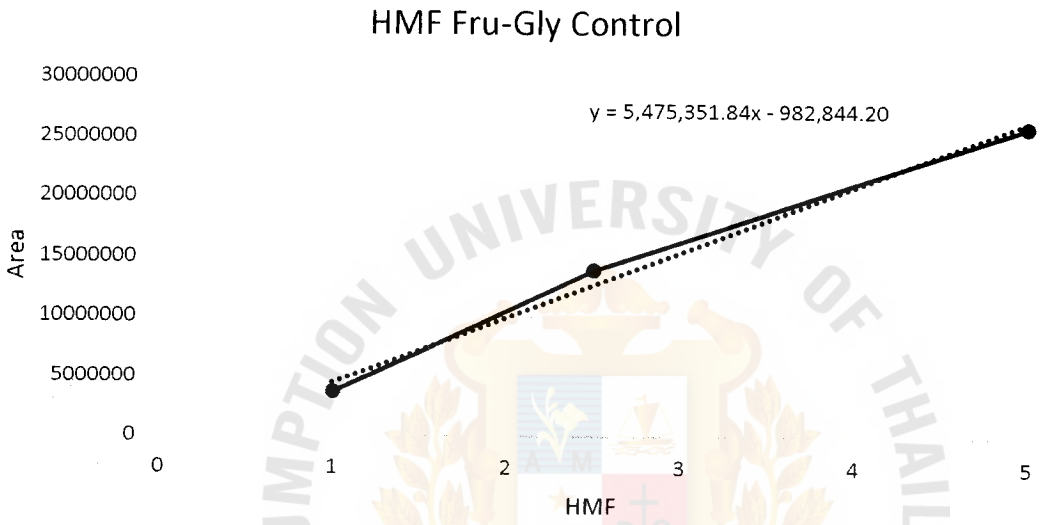


Glu-Lys Cats Whisker Conc 0.5					Glu-Lys Cats Whisker Conc 0.75				
Time	OD Values (420nm)			Average	Time	OD Values (420nm)			Average
1	0.023	0.019	0.027	0.023	1	-0.043	0.048	0.031	0.012
2	0.04	0.055	0.041	0.045	2	0.017	0.039	0.031	0.029
3	0.066	0.05	0.114	0.077	3	-0.07	0.098	0.074	0.034
4	0.157	0.179	0.253	0.196	4	0.101	-0.024	0.012	0.030
5	0.11	0.169	0.081	0.120	5	0.034	0.045	0.077	0.052
6	0.044	0.11	0.077	0.077	6	0.05	0.066	0.05	0.055
7	0.16	0.148	0.148	0.152	7	0.07	0.065	0.068	0.068

Glu-Lys Cats Whisker Conc 1.0				
Time	OD Values (420nm)			Average
1	0.029	0.016	0.022	0.022
2	0.043	0.026	0.109	0.059
3	0.092	0.062	0.063	0.072
4	0.13	0.176	0.171	0.159
5	0.153	0.067	0.067	0.096
6	0.155	0.154	0.12	0.143
7	0.184	0.069	0.093	0.115

HMF

1. Fructose – Glycine control
   HMF Slope

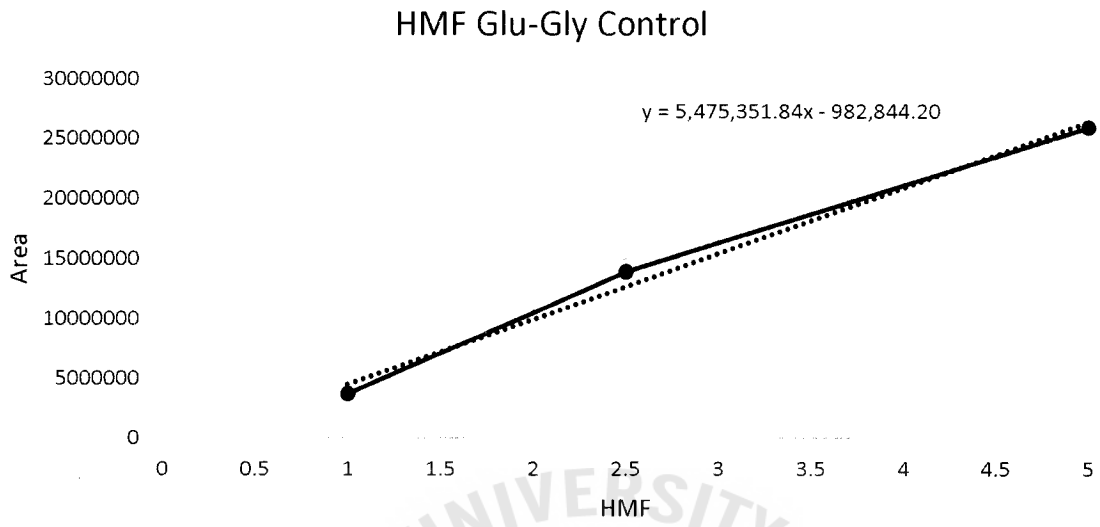


HMF (µg)	Area
1 µg	3719346
2.5 µg	13942594
5 µg	25930018

Y=	5,475,351.84x - 982,844.20
Slope	5,475,351.84
C	- 982,844.20

Glucose – Glycine control

HMF Slope

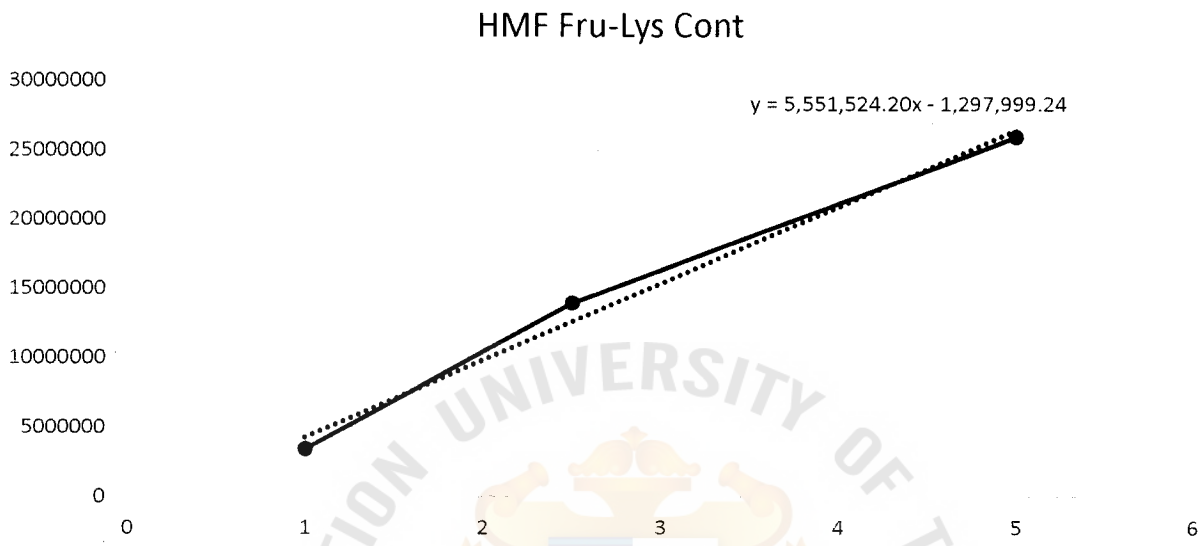


HMF (µg)	Area
1 µg	3719346
2.5 µg	13942594
5 µg	25930018

Y=	5,475,351.84x - 982,844.20
Slope	5,475,351.84
C	- 982,844.20

Fructose – Lysine Control

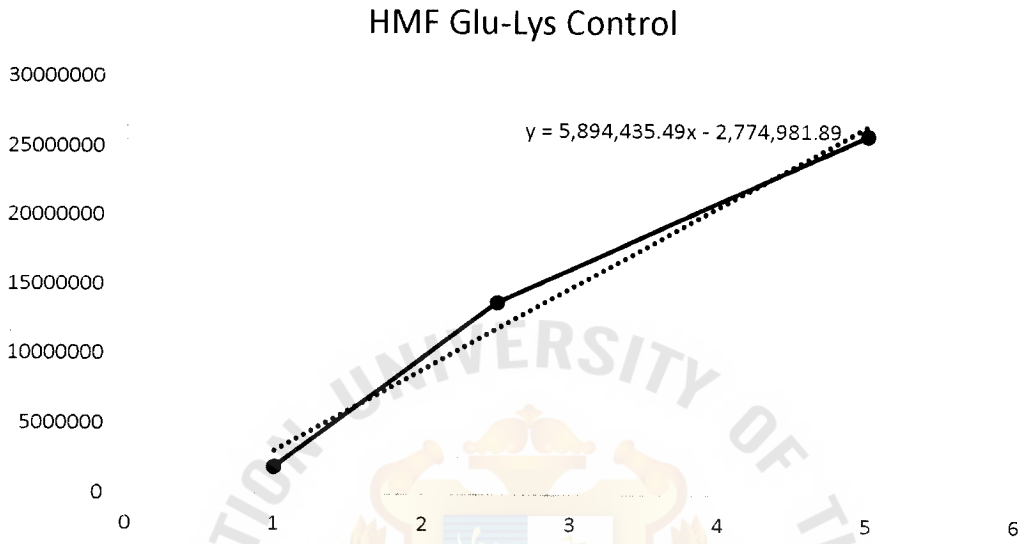
HMF Slope



HMF (µg)	Area
1 µg	3401422
2.5 µg	13944176
5 µg	25948360

Y=	5,551,524.20x - 1,297,999.24
Slope	5,551,524.20
C	- 1,297,999.24

Glucose-Lysine Control
HMF Slope



HMF (µg)	Area
1 µg	1952066
2.5 µg	13828927
5 µg	25996763

Y=	5,894,435.49x - 2,774,981.89
Slope	5,894,435.49x
C	- 2,774,981.89

HMF Calculations

Y=mx+C , where Y= area, m= slope

HMF= ((area-C)/m)/20unit, unit=mg/ml

HMF, Fructose – Glycine Control

Slope (m) = 5,475,351.84 C= - 982,844.20

Hour	Area(y)	Absolute(x)	mg/ml	µg/ml	mean	SD	mean±SD
1	14789	0.182204401	0.00911	9.1102	9.0951	0.0162933	9.0788
1	10653	0.181449015	0.009072	9.0725			
1	13944	0.182050073	0.009103	9.1025			
2	10702	0.181457965	0.009073	9.0729	9.1034	0.0302860	9.0731
2	18566	0.182894219	0.009145	9.1447			
2	12869	0.181853738	0.009093	9.0927			
3	3392	0.180122891	0.009006	9.0061	9.0054	0.0060032	8.9994
3	2467	0.179953952	0.008998	8.9977			
3	4071	0.180246901	0.009012	9.0123			
4	12192	0.181730093	0.009087	9.0865	9.0853	0.0113478	9.0739
4	13508	0.181970443	0.009099	9.0985			
4	10473	0.181416141	0.009071	9.0708			
5	12072	0.181708177	0.009085	9.0854	9.0882	0.0022744	9.0859
5	12385	0.181765342	0.009088	9.0883			
5	12682	0.181819585	0.009091	9.0910			
6	14153	0.182088244	0.009104	9.1044	9.1004	0.0040500	9.0963
6	13266	0.181926245	0.009096	9.0963			
6							
7	11113	0.181533028	0.009077	9.0767	9.0767	0.0063862	9.0703
7	10264	0.18137797	0.009069	9.0689			
7	11977	0.181690826	0.009085	9.0845			

HMF, Glucose – Glycine Control

Slope (m) = 5,475,351.84 C= - 982,844.20

Hour	Area(y)	Absolute(x)	mg/ml	µg/ml	mean	SD	mean±SD
1	1266	0.179734605	0.008987	8.9867	8.9924	0.0041	8.9883
1	2331	0.179929113	0.008996	8.9965			
1	2054	0.179878523	0.008994	8.9939			
2	2134	0.179893134	0.008995	8.9947	9.0002	0.0039	8.9962
2	2985	0.180048557	0.009002	9.0024			
2	3092	0.1800681	0.009003	9.0034			
3	3942	0.180223341	0.009011	9.0112	9.0016	0.0067	8.9949
3	2341	0.179930939	0.008997	8.9965			
3	2414	0.179944272	0.008997	8.9972			
4	3247	0.180096408	0.009005	9.0048	8.9977	8.9977	8.9977
4	1698	0.179813504	0.008991	8.9907			
5	2413	0.179944089	0.008997	8.9972	9.0011	0.0086	8.9924
5	1946	0.179858798	0.008993	8.9929			
2	4146	0.180260599	0.009013	9.0130			
6	2105	0.179887837	0.008994	8.9944	8.9951	0.0039	8.9913
6	2739	0.180003629	0.009	9.0002			
6	1711	0.179815878	0.008991	8.9908			
7	1012	0.179688215	0.008984	8.9844	8.9935	0.0104	8.9831
7	1404	0.179759809	0.008988	8.9880			
7	3603	0.180161427	0.009008	9.0081			

HMF, Fructose – Lysine Control

Slope (m) = 5,551,524.20 C= - 1,297,999.24

Hour	Area(y)	Absolute(x)	mg/ml	µg/ml	mean	SD	mean±SD
1	2297	0.234223286	0.011711	11.7112	11.7040	0.0050976	11.6989
1	1046	0.233997942	0.0117	11.6999			
1	1154	0.234017397	0.011701	11.7009			
2	1297	0.234043155	0.011702	11.7022	11.7062	0.0062844	11.6999
2	2735	0.234302183	0.011715	11.7151			
2	1216	0.234028565	0.011701	11.7014			
3	1292	0.234042255	0.011702	11.7021	11.7030	0.0013940	11.7016
3	1606	0.234098816	0.011705	11.7049			
3	1265	0.234037391	0.011702	11.7019			
4	4954	0.234701893	0.011735	11.7351	11.7256	0.0071572	11.7184
4	3703	0.23447655	0.011724	11.7238			
4	3037	0.234356583	0.011718	11.7178			
5	1353	0.234053243	0.011703	11.7027	11.7077	0.0058564	11.7019
5	2828	0.234318935	0.011716	11.7159			
2	1570	0.234092331	0.011705	11.7046			
6	2088	0.234185639	0.011709	11.7093	11.7073	0.0027328	11.7045
6	1436	0.234068193	0.011703	11.7034			
6	2071	0.234182576	0.011709	11.7091			
7	2212	0.234207975	0.01171	11.7104	11.7108	0.0007283	11.7100
7	2178	0.23420185	0.01171	11.7101			
7	2364	0.234235355	0.011712	11.7118			

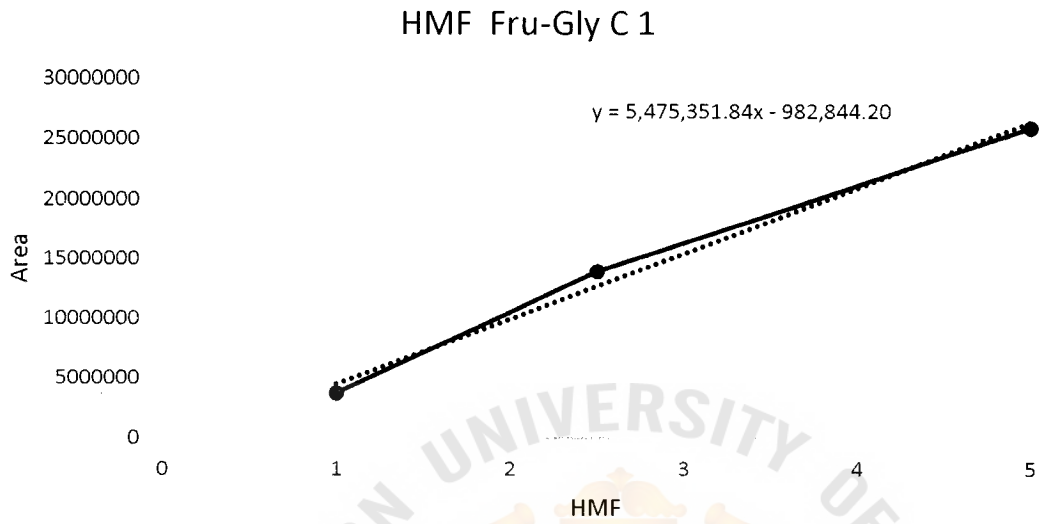
HMF, Glucose – Lysine Control

Slope (m) = 5,894,435.49 C= - 2,774,981.89

Hour	Area(y)	Absolute(x)	mg/ml	µg/ml	mean	SD	mean±SD
1	4539	0.471549972	0.023577	23.5775	23.5763	0.0034896	23.5728
1	3831	0.471429859	0.023571	23.5715			
1	4806	0.471595269	0.02358	23.5798			
2	3220	0.471326202	0.023566	23.5663	23.5753	0.0149685	23.5603
2	2849	0.471263261	0.023563	23.5632			
2	6764	0.471927447	0.023596	23.5964			
3	13567	0.473081586	0.023654	23.6541	23.6186	0.0263236	23.5923
3	8462	0.472215515	0.023611	23.6108			
3	6137	0.471821075	0.023591	23.5911			
4	4303	0.471509934	0.023575	23.5755	23.5950	0.0205565	23.5745
4	5561	0.471723356	0.023586	23.5862			
4	9956	0.472468974	0.023623	23.6234			
5	4786	0.471591876	0.02358	23.5796	23.6081	0.0376680	23.5704
5	5234	0.47166788	0.023583	23.5834			
2	14422	0.473226638	0.023661	23.6613			
6	5969	0.471792574	0.02359	23.5896	23.6035	0.0116015	23.5919
6	9316	0.472360397	0.023618	23.6180			
6	7517	0.472055194	0.023603	23.6028			
7	14632	0.473262265	0.023663	23.6631	23.6231	0.0342344	23.5888
7	10332	0.472532763	0.023627	23.6266			
7	4773	0.47158967	0.023579	23.5795			

HMF Slope with Best conditions

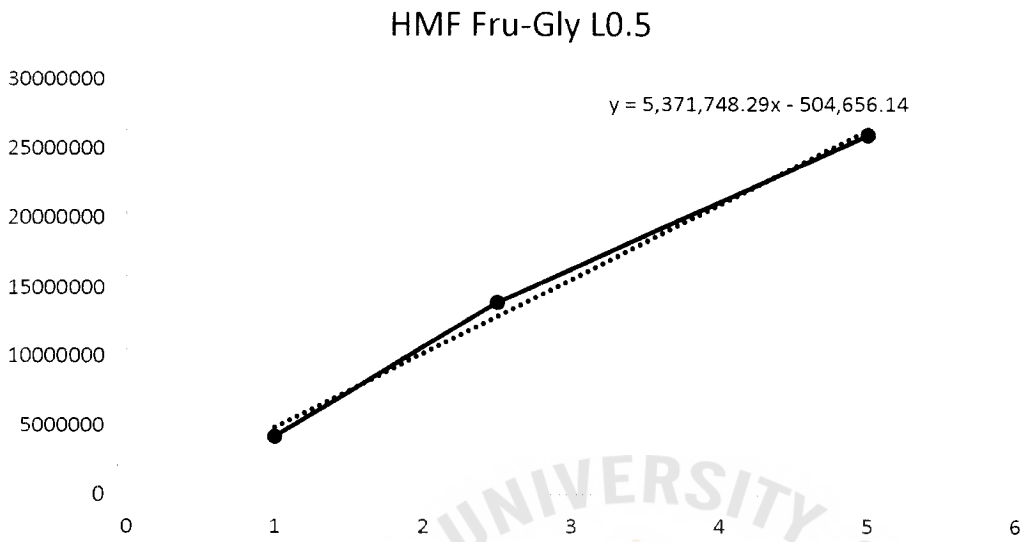
Fructose-Glycine Cat's whisker Conc. 1.0



HMF(µg)	Area
1 µg	3719346
2.5 µg	13942594
5 µg	25930018

Y=	5,475,351.84x - 982,844.20
Slope	5,475,351.84
C	- 982,844.20

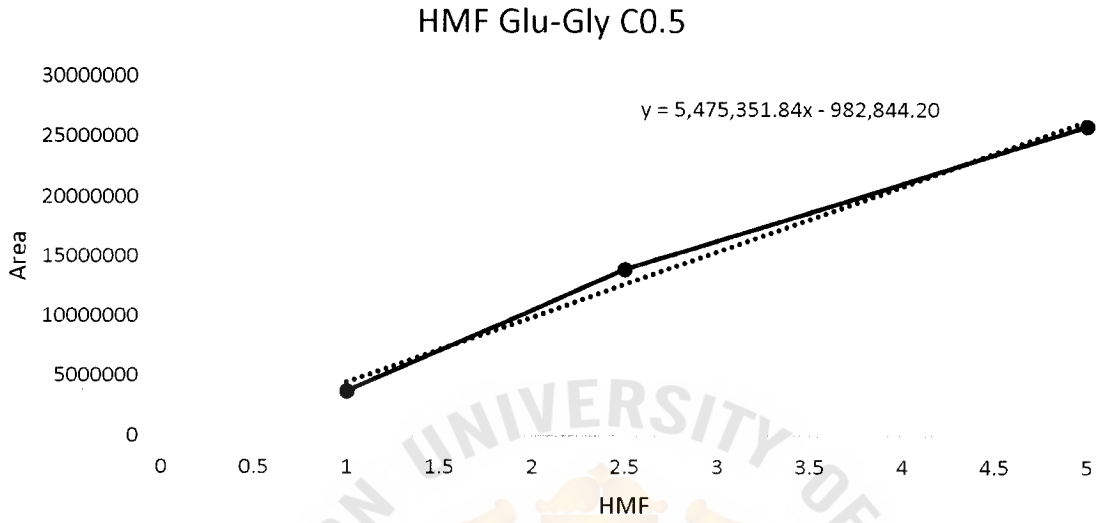
Fructose-Glycine Laurel Vine tea Conc. 0.5



HMF(µg)	Area
1 µg	4219685
2.5 µg	13960566
5 µg	25965641

Y=	5,371,748.29x - 504,656.14
Slope	5,371,748.29
C	- 504,656.14

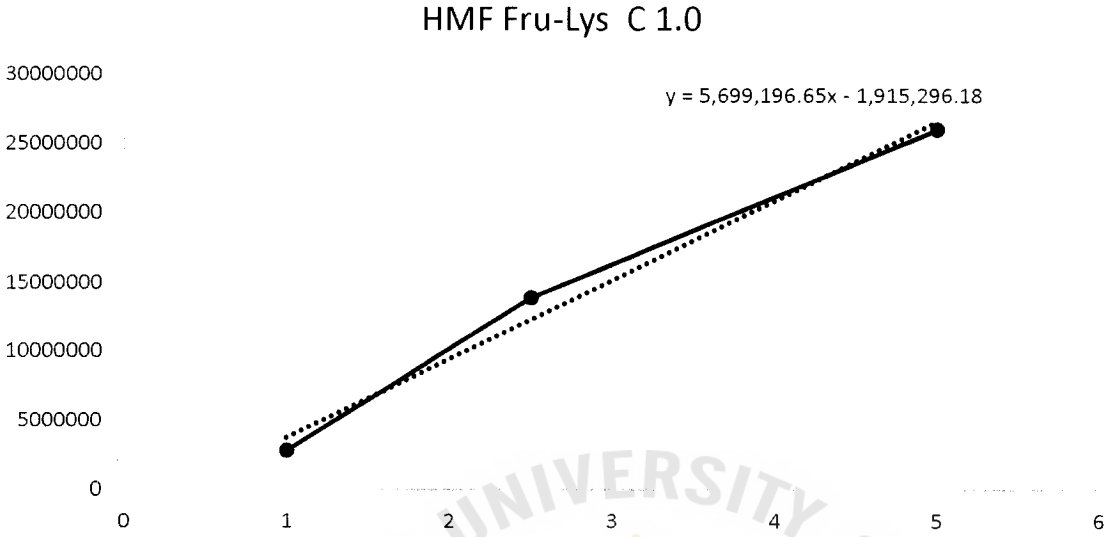
Glucose-Glycine Cat's Whisker Conc 0.5



HMF (µg)	Area
1 µg	3719346
2.5 µg	13942594
5 µg	25930018

Y=	5,475,351.84x - 982,844.20
Slope	5,475,351.84
C	- 982,844.20

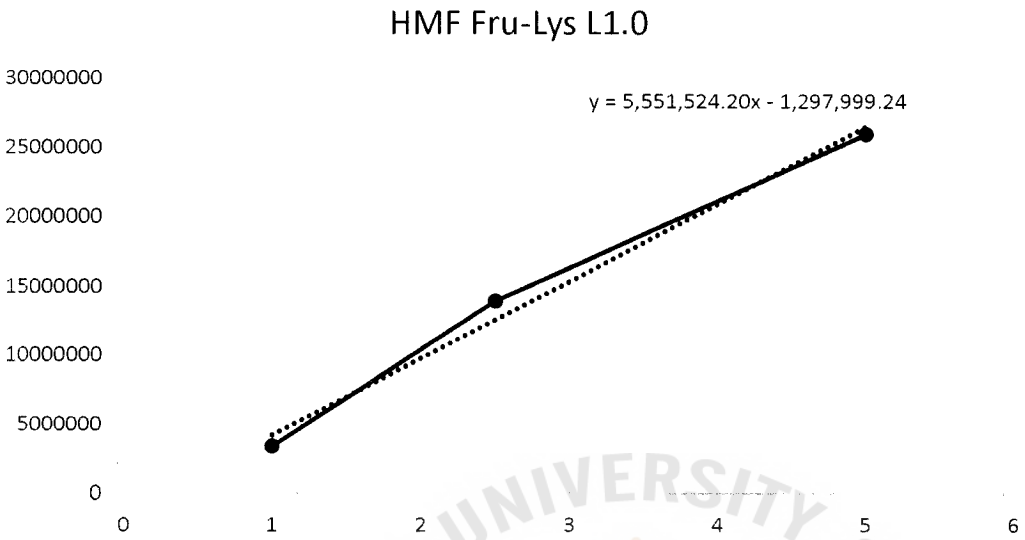
Fructose-Lysine Cat's whisker Conc 1.0



HMF (µg)	Area
1 µg	2796032
2.5 µg	13913285
5 µg	25987966

Y=	5,699,196.65x - 1,915,296.18
Slope	5,699,196.65
C	- 1,915,296.18

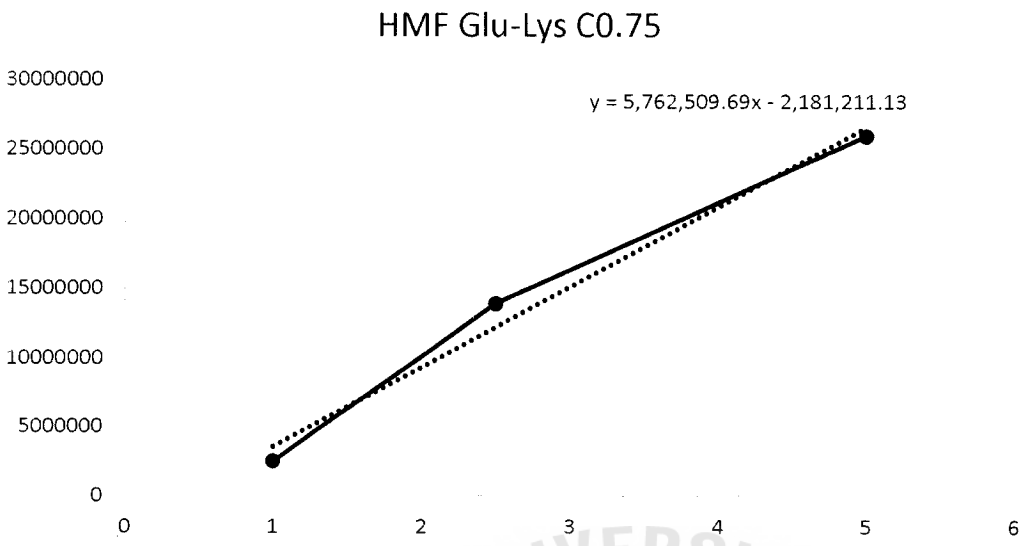
Fructose-Lysine Laurel Vine Tea Conc 1.0



HMF (µg)	Area
1 µg	3401422
2.5 µg	13944176
5 µg	25948360

Y=	5,551,524.20x - 1,297,999.24
Slope	5,551,524.20
C	- 1,297,999.24

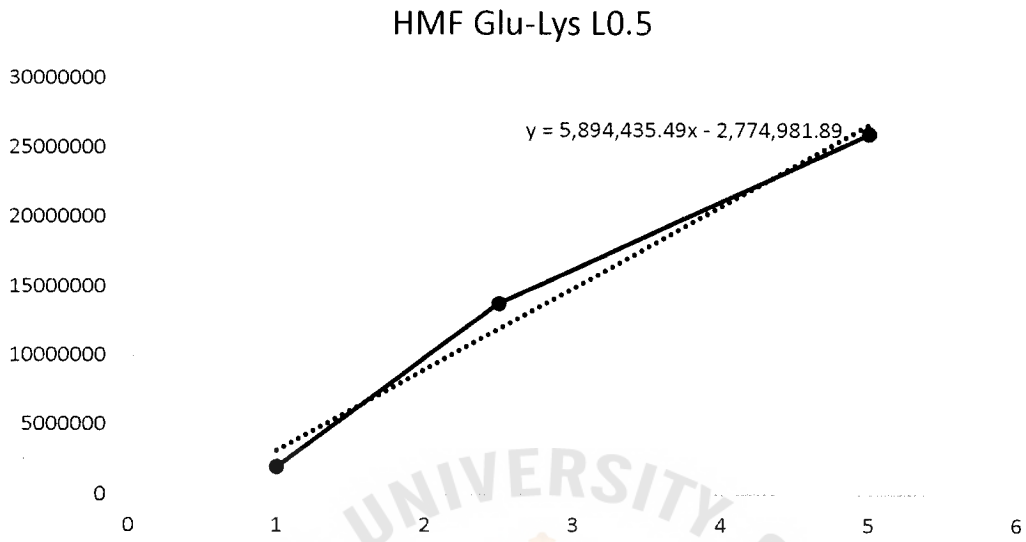
Glucose-Lysine Cats whisker Conc 0.75



HMF (µg)	Area
1 µg	2534593
2.5 µg	13899792
5 µg	26003314

Y=	5,762,509.69x - 2,181,211.13
Slope	5,762,509.69
C	- 2,181,211.13

Glucose-Lysine L0.5



HMF (µg)	Area
1 µg	1952066
2.5 µg	13828927
5 µg	25996763

Y=	5,894,435.49x - 2,774,981.89
Slope	5,894,435.49
C	- 2,774,981.89

Best Conditions ; HMF Calculations

Y=mx+C , where Y= area, m= slope
HMF= ((area-C)/m)/20unit, unit=mg/ml

HMF, Fructose – Glycine C1.0

Slope (m) = 5,475,351.84 C= - 982,844.20

Area(y)	Absolute(x)	mg/ml	µg/ml	mean	SD	mean±SD
11956	0.181686991	0.009084	9.0843	26.1205	12.0463861	14.0741
13133	0.69274377	0.034637	34.6372			
13757	0.692799959	0.03464	34.6400			
16981	0.69309027	0.034655	34.6545	34.6448	0.0089949	34.6358
15298	0.692938722	0.034647	34.6469			
12160	0.692656155	0.034633	34.6328			
12554	0.692691633	0.034635	34.6346	34.6513	0.0135413	34.6378
19920	0.693354918	0.034668	34.6677			
16348	0.693033271	0.034652	34.6517			
11025	0.692553952	0.034628	34.6277	34.6232	0.0159329	34.6073
13779	0.69280194	0.03464	34.6401			
5284	0.692036993	0.034602	34.6018			
7415	0.692228883	0.034611	34.6114	34.5930	0.0130307	34.5800
1262	0.691674825	0.034584	34.5837			
1289	0.691677256	0.034584	34.5839			
8213	0.69230074	0.034615	34.6150	34.6192	0.0042665	34.6149
8746	0.692348735	0.034617	34.6174			
10436	0.692500914	0.034625	34.6250			
7970	0.692278859	0.034614	34.6139	34.6095	0.0105326	34.5990
3752	0.691899041	0.034595	34.5950			
9220	0.692391417	0.03462	34.6196			

HMF, Fructose – Glycine L0.5

Slope (m) = 5,371,748.29 C= - 504,656.14

Hour	Area(y)	Absolute(x)	mg/ml	µg/ml	mean	SD	mean±SD
1	8373	0.095505059	0.004775	4.7753	4.7598	0.0117933	4.7480
1	6466	0.095150054	0.004758	4.7575			
1	5299	0.094932806	0.004747	4.7466			
2	7946	0.09542557	0.004771	4.7713	4.7696	0.0026040	4.7670
2	7985	0.09543283	0.004772	4.7716			
2	7373	0.0953189	0.004766	4.7659			
3	21072	0.097869094	0.004893	4.8935	4.8934	0.0037772	4.8897
3	21565	0.097960871	0.004898	4.8980			
3	20571	0.097775829	0.004889	4.8888			
4	18604	0.097409654	0.00487	4.8705	4.8542	0.0129490	4.8413
4	16765	0.097067307	0.004853	4.8534			
4	15200	0.096775968	0.004839	4.8388			
5	18965	0.097476857	0.004874	4.8738	4.8711	0.0076103	4.8635
5	17553	0.097214	0.004861	4.8607			
2	19489	0.097574404	0.004879	4.8787			
6	8086	0.095451632	0.004773	4.7726	4.7624	0.0160167	4.7464
6	4560	0.094795235	0.00474	4.7398			
6	8323	0.095495752	0.004775	4.7748			
7	7251	0.095296189	0.004765	4.7648	4.7621	0.0157979	4.7463
7	8880	0.095599442	0.00478	4.7800			
7	4753	0.094831163	0.004742	4.7416			

HMF, Glucose – Glycine C0.5

Slope (m) = 5,475,351.84 C= - 982,844.20

Hour	Area(y)	Absolute(x)	mg/ml	µg/ml	mean	SD	mean±SD
1	2587	0.179975868	0.008999	8.9988	8.9950	0.0034958	8.9915
1	2274	0.179918703	0.008996	8.9959			
1	1665	0.179807477	0.00899	8.9904			
2	2408	0.179943176	0.008997	8.9972	8.9911	0.0043532	8.9867
2	1513	0.179779716	0.008989	8.9890			
2	1311	0.179742824	0.008987	8.9871			
3	2703	0.179997054	0.009	8.9999	8.9943	0.0039923	8.9904
3	1915	0.179853136	0.008993	8.9927			
3	1681	0.179810399	0.008991	8.9905			
4	1844	0.179840169	0.008992	8.9920	8.9946	0.0042459	8.9904
4	2789	0.180012761	0.009001	9.0006			
4	1766	0.179825923	0.008991	8.9913			
5	3216	0.180090746	0.009005	9.0045	9.0036	0.0022518	9.0014
5	2780	0.180011117	0.009001	9.0006			
2	3360	0.180117046	0.009006	9.0059			
6	4127	0.180257128	0.009013	9.0129	9.0073	0.0039599	9.0033
6	3223	0.180092025	0.009005	9.0046			
6	3192	0.180086363	0.009004	9.0043			
7	3787	0.180195032	0.00901	9.0098	8.9990	0.0076274	8.9914
7	2104	0.179887654	0.008994	8.9944			
7	1938	0.179857337	0.008993	8.9929			

HMF, Fructose- Lysine C1

Slope (m) = 5,699,196.65x C= - 1,915,296.18

Hour	Area(y)	Absolute(x)	µg/µl	mg/ml	mean	SD	mean±SD
1	11555	0.338091717	0.016905	16.9046	16.9148	0.0100102	16.9047
1	12322	0.338226297	0.016911	16.9113			
1	14266	0.338567398	0.016928	16.9284			
2	17777	0.33918345	0.016959	16.9592	16.9539	0.0069065	16.9470
2	16068	0.338883583	0.016944	16.9442			
2	17696	0.339169237	0.016958	16.9585			
3	13141	0.338370002	0.016919	16.9185	16.9571	0.0303152	16.9268
3	21583	0.339851263	0.016993	16.9926			
3	17891	0.339203452	0.01696	16.9602			
4	12127	0.338192082	0.01691	16.9096	16.9226	0.0125347	16.9101
4	15540	0.338790938	0.01694	16.9395			
4	13163	0.338373862	0.016919	16.9187			
5	8678	0.337586909	0.016879	16.8793	16.8370	0.0299328	16.8071
5	1603	0.336345506	0.016817	16.8173			
2	1288	0.336290235	0.016815	16.8145			
6	8595	0.337572345	0.016879	16.8786	16.8895	0.0118820	16.8776
6	9199	0.337678325	0.016884	16.8839			
6	11722	0.338121019	0.016906	16.9061			
7	8319	0.337523918	0.016876	16.8762	16.8510	0.0178226	16.8332
7	4146	0.336791709	0.01684	16.8396			
7	3885	0.336745913	0.016837	16.8373			

HMF, Fructose- Lysine L1

Slope (m) = 5,551,524.20 C= -1,297,999.24

Hour	Area(y)	Absolute(x)	mg/ml	µg/ml	mean	SD	mean±SD
1	3485	0.234397292	0.01172	11.7199	11.7151	0.0050920	11.7101
1	2176	0.234161501	0.011708	11.7081			
1	3222	0.234349918	0.011717	11.7175			
2	2831	0.234279487	0.011714	11.7140	11.7157	0.0069173	11.7087
2	4039	0.234497085	0.011725	11.7249			
2	2186	0.234163302	0.011708	11.7082			
3	1117	0.233970743	0.011699	11.6985	11.6990	0.0010048	11.6980
3	1063	0.233961016	0.011698	11.6981			
3	1322	0.234007669	0.0117	11.7004			
4	1942	0.234119351	0.011706	11.7060	11.7024	0.0026015	11.6998
4	1449	0.234030546	0.011702	11.7015			
4	1256	0.233995781	0.0117	11.6998			
5	2262	0.234176992	0.011709	11.7088	11.7031	0.0045874	11.6985
5	1584	0.234054864	0.011703	11.7027			
2	1016	0.233952549	0.011698	11.6976			
6	2215	0.234168526	0.011708	11.7084	11.7050	0.0037189	11.7013
6	2026	0.234134481	0.011707	11.7067			
6	1260	0.233996501	0.0117	11.6998			
7	1713	0.234078101	0.011704	11.7039	11.7010	0.0021288	11.6988
7	1160	0.233978488	0.011699	11.6989			
7	1288	0.234001545	0.0117	11.7001			

HMF, Fructose- Glycine C0.75

Slope (m) = 5,762,509.69 C= - 2,181,211.13

Hour	Area(y)	Absolute(x)	mg/ml	µg/ml	mean	SD	mean±SD
1	1195	0.378724939	0.018936	18.9362	18.9372	0.0011421	18.9361
1	1494	0.378776826	0.018939	18.9388			
1	1240	0.378732748	0.018937	18.9366			
2	1301	0.378743334	0.018937	18.9372	18.9393	0.0014984	18.9378
2	1676	0.378808409	0.01894	18.9404			
2	1658	0.378805286	0.01894	18.9403			
3	5092	0.379401207	0.01897	18.9701	18.9746	0.0045501	18.9700
3	6331	0.379616217	0.018981	18.9808			
3	5418	0.379457779	0.018973	18.9729			
4	1360	0.378753572	0.018938	18.9377	18.9440	0.0093083	18.9347
4	1294	0.378742119	0.018937	18.9371			
4	3602	0.379142639	0.018957	18.9571			
5	1194	0.378724765	0.018936	18.9362	18.9353	0.0006564	18.9347
5	1063	0.378702032	0.018935	18.9351			
2	1015	0.378693702	0.018935	18.9347			
6	1247	0.378733963	0.018937	18.9367	18.9358	0.0006150	18.9352
6	1103	0.378708974	0.018935	18.9354			
6	1091	0.378706891	0.018935	18.9353			
7	1192	0.378724418	0.018936	18.9362	18.9384	0.0029385	18.9355
7	1220	0.378729277	0.018936	18.9365			
7	1924	0.378851446	0.018943	18.9426			

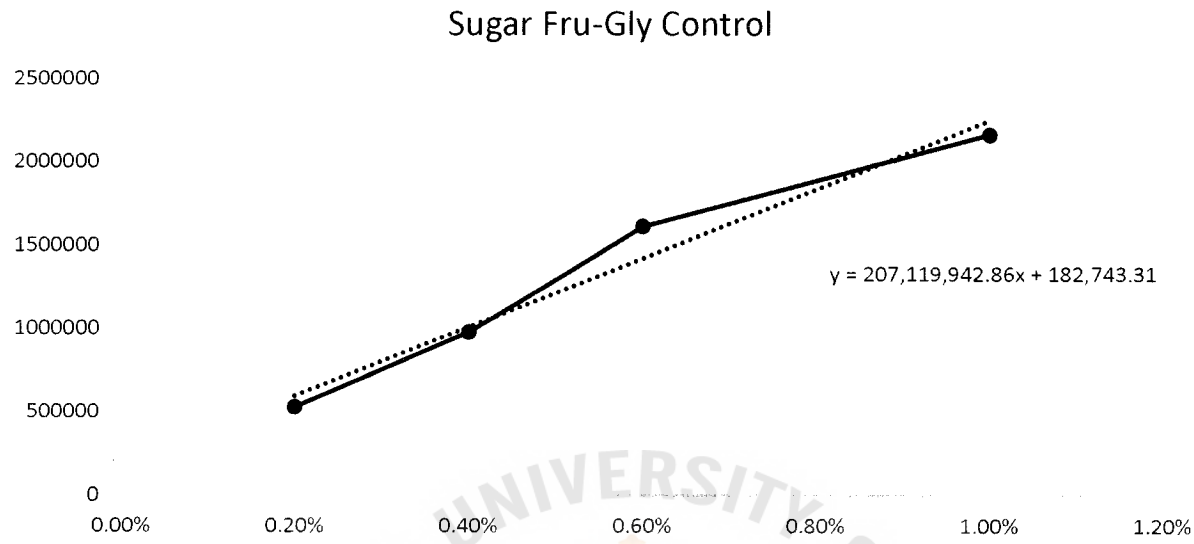
HMF, Fructose- Glycine L0.5

Slope (m) = 5,894,435.49 C= - 2,774,981.89

Hour	Area(y)	Absolute(x)	mg/ml	µg/ml	mean	SD	mean±SD
2	7127	0.47198903	0.023599	23.5995	23.5919	0.0075834	23.5843
2	5339	0.471685693	0.023584	23.5843			
3	9359	0.472367692	0.023618	23.6184	23.6262	0.0060400	23.6202
3	11092	0.472661698	0.023633	23.6331			
3	10396	0.472543621	0.023627	23.6272			
4	23319	0.474736028	0.023737	23.7368	23.7186	0.0257818	23.6928
4	23328	0.474737555	0.023737	23.7369			
4	16876	0.473642963	0.023682	23.6821			
5	9930	0.472464563	0.023623	23.6232	23.6110	0.0122743	23.5987
2	7036	0.471973592	0.023599	23.5987			
6	2731	0.471243242	0.023562	23.5622	23.5581	0.0040674	23.5540
6	1772	0.471080546	0.023554	23.5540			
7	38574	0.477324062	0.023866	23.8662	23.7521	0.1140949	23.6380
7	11673	0.472760266	0.023638	23.6380			

SUGAR CONTENTS

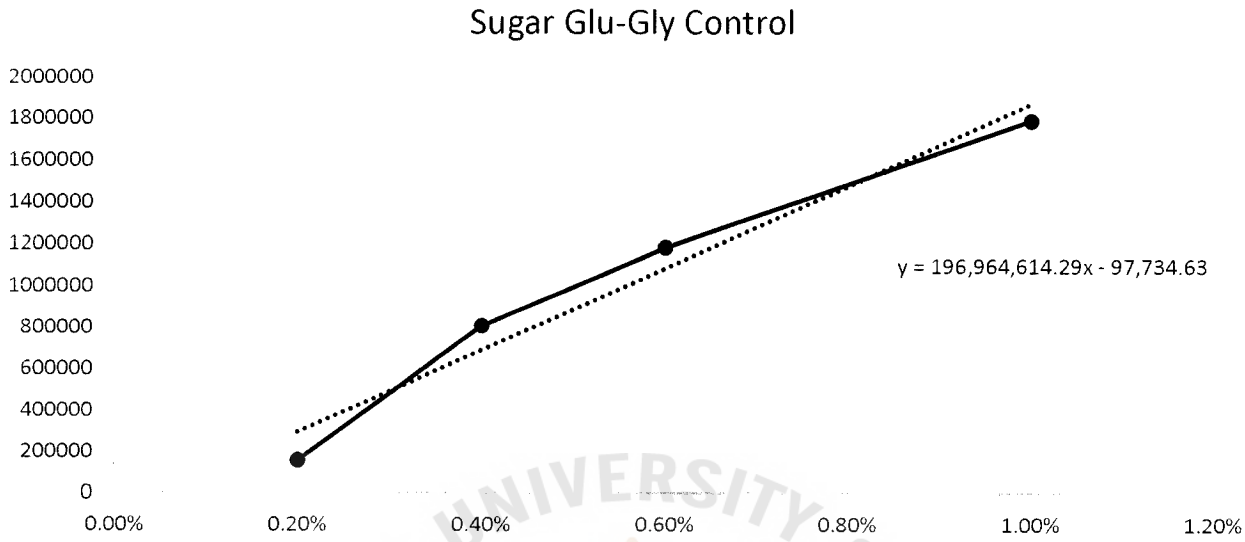
Fructose-Glycine Control



Sugar (%)	Area
0.2	527364
0.4	977716
0.6	1614962
1	2167570

Y=	207,119,942.86x + 182,743.31
Slope	207,119,942.86
C	182,743.31

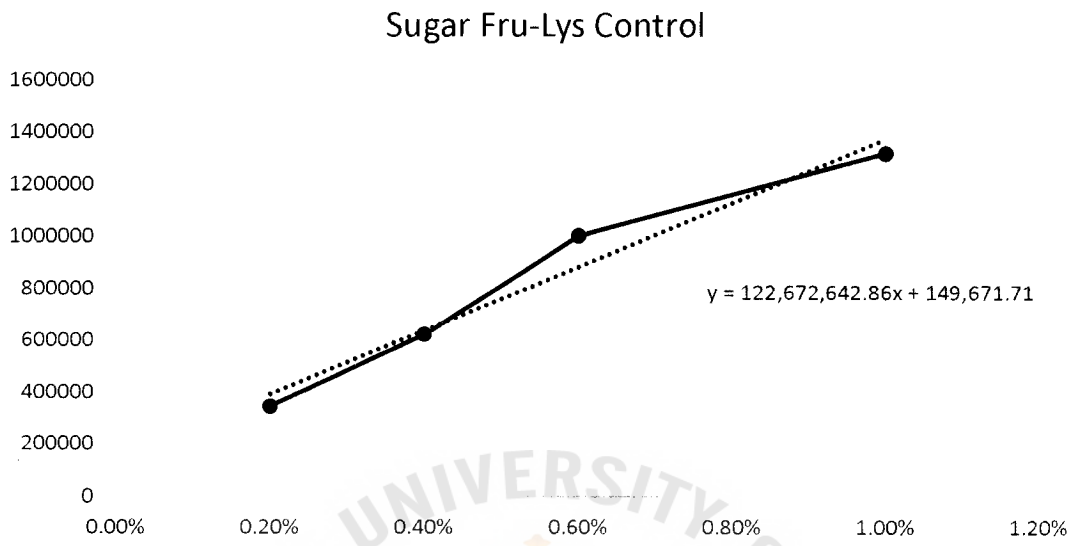
Glucose-Glycine Control



Sugar (%)	Area
0.2	157648
0.4	808012
0.6	1184314
1	1792309

Y=	196,964,614.29x - 97,734.63
Slope	196,964,614.29
C	- 97,734.63

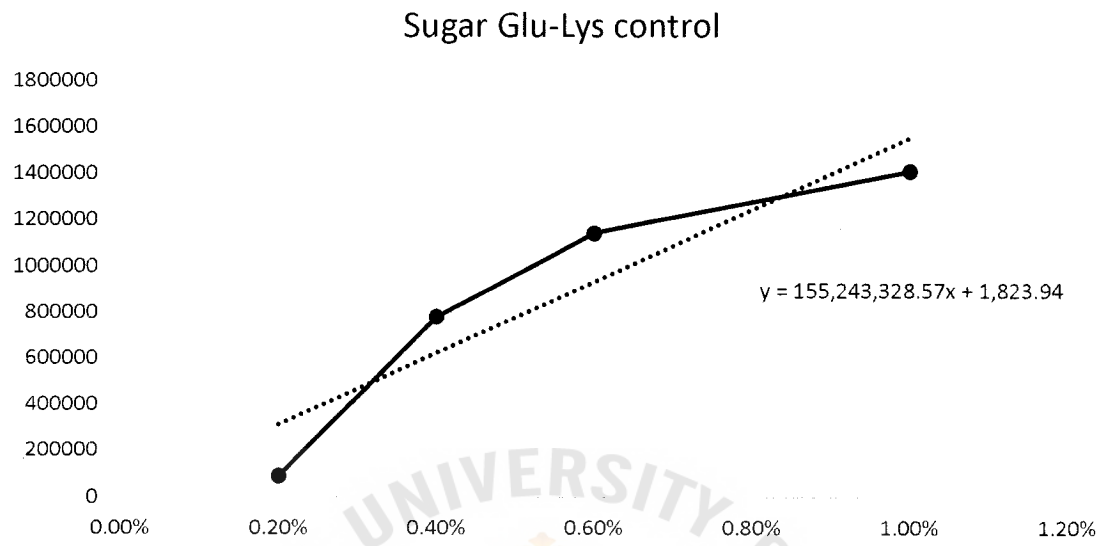
Fructose-Lysine Control



Sugar (%)	Area
0.2	347741
0.4	624408
0.6	1004191
1	1321145

Y=	122,672,642.86x + 149,671.71
Slope	122,672,642.86
C	149,671.71

Glucose-Lysine Control



Sugar (%)	Area
0.2	89361
0.4	780350
0.6	1142854
1	1410084

Y=	155,243,328.57x + 1,823.94
Slope	155,243,328.57
C	1,823.94

Sugar Content Calculations

Y=mx+C , where Y= area, m= slope
HMF= ((area-C)/m)/20unit, unit=mg/ml

Sugar, Fructose – Glycine Control

Slope (m) = 207,119,942.86 C= 182,743.31

Hour	Area	Absolute (x)	mg/ml	µg/ml	mean	SD	mean±SD
1	2652784	0.0119	0.0006	0.5963	0.6347	0.0416	0.5931
1	3050970	0.0138	0.0007	0.6924			
1	2731631	0.0123	0.0006	0.6153			
2	2078972	0.0092	0.0005	0.4578	0.6219	0.1179	0.5041
2	3055465	0.0139	0.0007	0.6935			
2	3142544	0.0143	0.0007	0.7145			
3	1579025	0.0067	0.0003	0.3371	0.3344	0.0235	0.3109
3	1443667	0.0061	0.0003	0.3044			
3	1681730	0.0072	0.0004	0.3619			
4	2067938	0.0091	0.0005	0.4551	0.4481	0.0276	0.4204
4	1886203	0.0082	0.0004	0.4112			
4	2162138	0.0096	0.0005	0.4778			
5	2151541	0.0095	0.0005	0.4753	0.4203	0.0455	0.3748
5	1689697	0.0073	0.0004	0.3638			
5	1929947	0.0084	0.0004	0.4218			
6	2082996	0.0092	0.0005	0.4587	0.4556	0.0032	0.4524
6	2056713	0.0090	0.0005	0.4524			
7	2102517	0.0093	0.0005	0.4634	0.4379	0.0181	0.4199
7	1940025	0.0085	0.0004	0.4242			
7	1948031	0.0085	0.0004	0.4262			

Sugar, Glucose – Glycine Control

Slope (m) = 196,964,614.29 C= -97,734.63

Hour	Area	Absolute (x)	mg/ml	µg/ml	mean	SD	mean±SD
1	2112834	0.0112	0.0006	0.5612	0.5782	0.0147	0.5635
1	2172411	0.0115	0.0006	0.5763			
1	2254064	0.0119	0.0006	0.5970			
2	2153811	0.0114	0.0006	0.5716	0.6383	0.0877	0.5506
2	2191507	0.0116	0.0006	0.5811			
2	2904419	0.0152	0.0008	0.7621			
3	2522133	0.0133	0.0007	0.6651	0.6804	0.0772	0.6032
3	2243939	0.0119	0.0006	0.5944			
3	2981182	0.0156	0.0008	0.7816			
4	3310851	0.0173	0.0009	0.8653	0.8311	0.0398	0.7914
4	2956586	0.0155	0.0008	0.7753			
4	3261729	0.0171	0.0009	0.8528			
5	1292614	0.0071	0.0004	0.3529	0.6949	0.2481	0.4468
5	3045227	0.0160	0.0008	0.7978			
5	3581450	0.0187	0.0009	0.9340			
6	2048993	0.0109	0.0005	0.5450	0.6442	0.1580	0.4862
6	3318619	0.0173	0.0009	0.8673			
6	1952499	0.0104	0.0005	0.5205			
7	1129912	0.0062	0.0003	0.3116	0.3275	0.0112	0.3162
7	1218529	0.0067	0.0003	0.3341			
7	1228162	0.0067	0.0003	0.3366			

Sugar, Fructose – Lysine Control

Slope (m) = 122,672,642.86 C= 149,671.71

Hour	Area	Absolute (x)	mg/ml	µg/ml	mean	SD	mean±SD
1	2726448	0.0210	0.0011	1.0503	1.3090	0.1917	1.1173
1	3506984	0.0274	0.0014	1.3684			
1	3850630	0.0302	0.0015	1.5085			
2	3676358	0.0287	0.0014	1.4374	1.2990	0.1021	1.1969
2	3254280	0.0253	0.0013	1.2654			
2	3079565	0.0239	0.0012	1.1942			
3	3338719	0.0260	0.0013	1.2998	1.0586	0.1794	0.8792
3	2284030	0.0174	0.0009	0.8699			
3	2618040	0.0201	0.0010	1.0061			
4	2738928	0.0211	0.0011	1.0554	0.9566	0.1036	0.8530
4	2605320	0.0200	0.0010	1.0009			
4	2145560	0.0163	0.0008	0.8135			
5	2657394	0.0204	0.0010	1.0221	0.9457	0.1340	0.8117
5	2007819	0.0151	0.0008	0.7574			
5	2744626	0.0212	0.0011	1.0577			
6	2413011	0.0185	0.0009	0.9225	0.9322	0.0402	0.8919
6	2329394	0.0178	0.0009	0.8884			
6	2567711	0.0197	0.0010	0.9856			
7	2510871	0.0192	0.0010	0.9624	1.0555	0.0808	0.9747
7	2712629	0.0209	0.0010	1.0446			
7	2994375	0.0232	0.0012	1.1595			

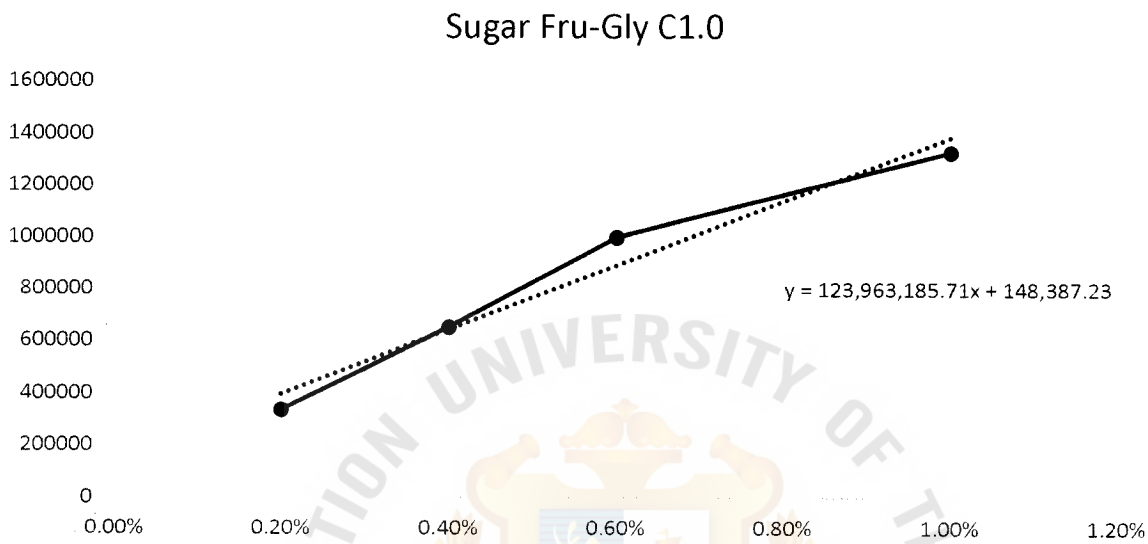
Sugar, Glucose – Lysine Control

Slope (m) = 155,243,328.57 C= 1,823.94

Hour	Area	Absolute (x)	mg/ml	µg/ml	mean	SD	mean±SD
1	1785264	0.0115	0.0006	0.5744	0.6818	0.0840	0.5978
1	2149373	0.0138	0.0007	0.6917			
1	2421966	0.0156	0.0008	0.7795			
2	1864057	0.0120	0.0006	0.5998	0.8668	0.1891	0.6777
2	3066929	0.0197	0.0010	0.9872			
2	3148367	0.0203	0.0010	1.0134			
3	2686871	0.0173	0.0009	0.8648	0.7635	0.0717	0.6918
3	2204405	0.0142	0.0007	0.7094			
3	2225696	0.0143	0.0007	0.7163			
4	2257713	0.0145	0.0007	0.7266	0.5573	0.1205	0.4368
4	1520889	0.0098	0.0005	0.4893			
4	1417449	0.0091	0.0005	0.4559			
5	1098703	0.0071	0.0004	0.3533	0.5898	0.3982	0.1916
5	825897	0.0053	0.0003	0.2654			
5	3574605	0.0230	0.0012	1.1507			
6	3141947	0.0202	0.0010	1.0114	1.0342	0.0519	0.9823
6	3435652	0.0221	0.0011	1.1060			
6	3060678	0.0197	0.0010	0.9852			
7	3466698	0.0223	0.0011	1.1159	1.0908	0.0218	1.0690
7	3397911	0.0219	0.0011	1.0938			
7	3301458	0.0213	0.0011	1.0627			

Sugar Content Slope with Best conditions

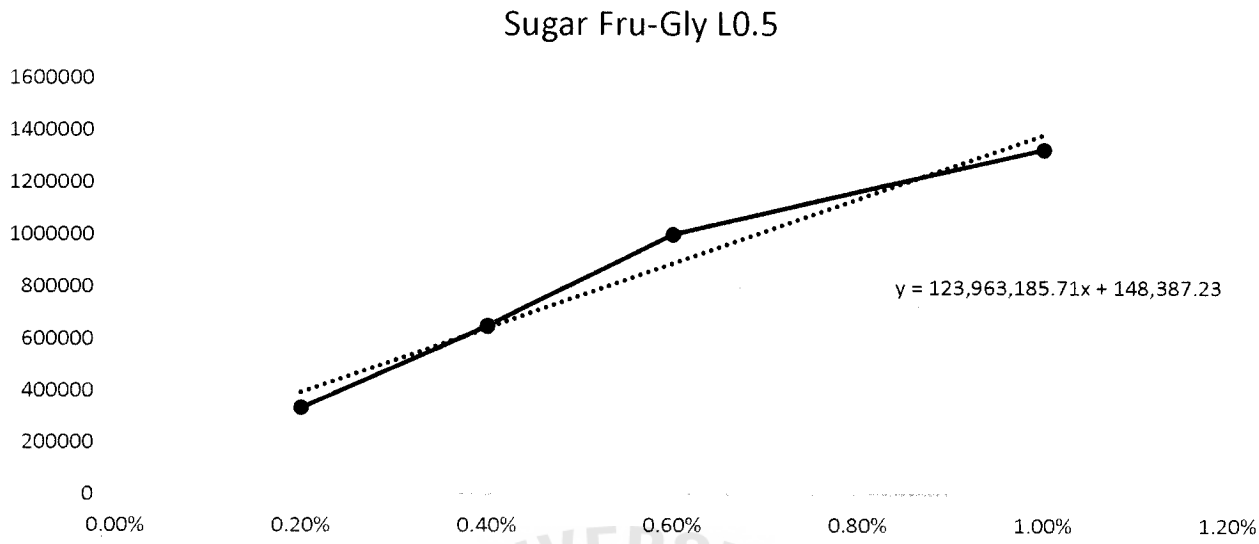
Fructose-Glycine C1



Sugar (%)	Area
0.2	335207
0.4	652575
0.6	1001877
1	1331080

Y=	123,963,185.71x + 148,387.23
Slope	123,963,185.71
C	148,387.23

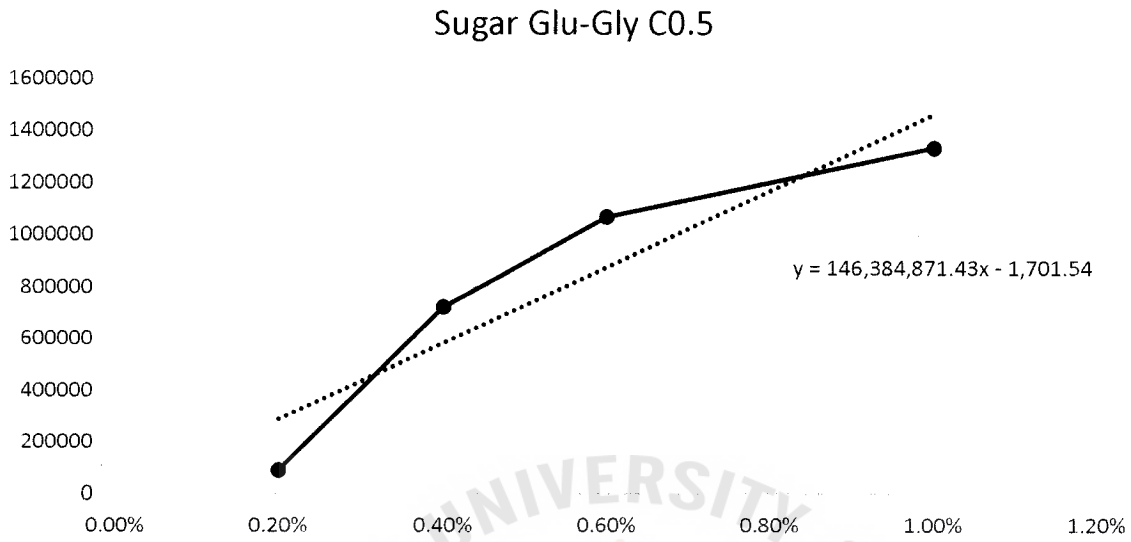
Fructose-Glycine L0.5



Sugar (%)	Area
0.2	335207
0.4	652575
0.6	1001877
1	1331080

Y=	123,963,185.71x + 148,387.23
Slope	123,963,185.71
C	148,387.23

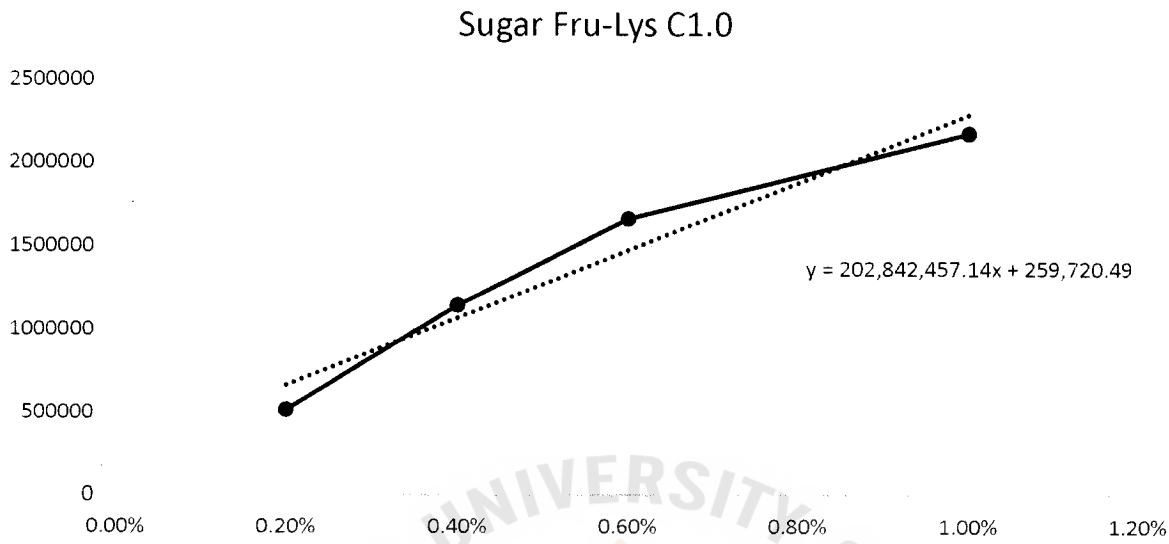
Glucose-Glysine C0.5



Sugar (%)	Area
0.2	92045
0.4	722074
0.6	1067300
1	1332242

Y=	146,384,871.43x - 1,701.54
Slope	146,384,871.43
C	- 1,701.54

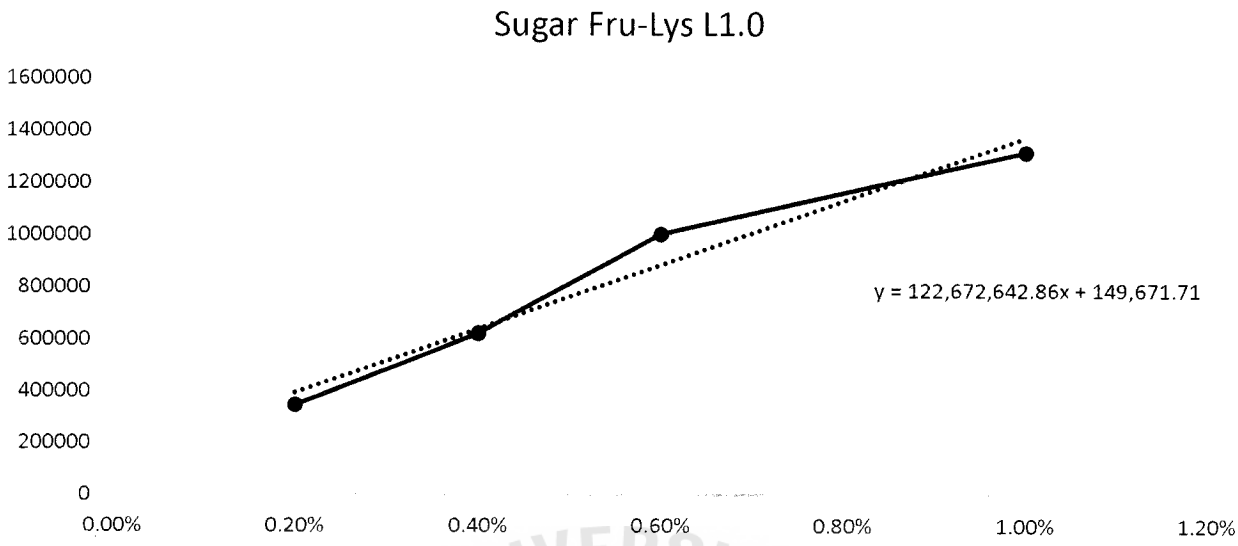
Fructose-Lysine C1.0



Sugar (%)	Area
0.2	515380
0.4	1147187
0.6	1662681
1	2176168

Y=	202,842,457.14x + 259,720.49
Slope	202,842,457.14
C	259,720.49

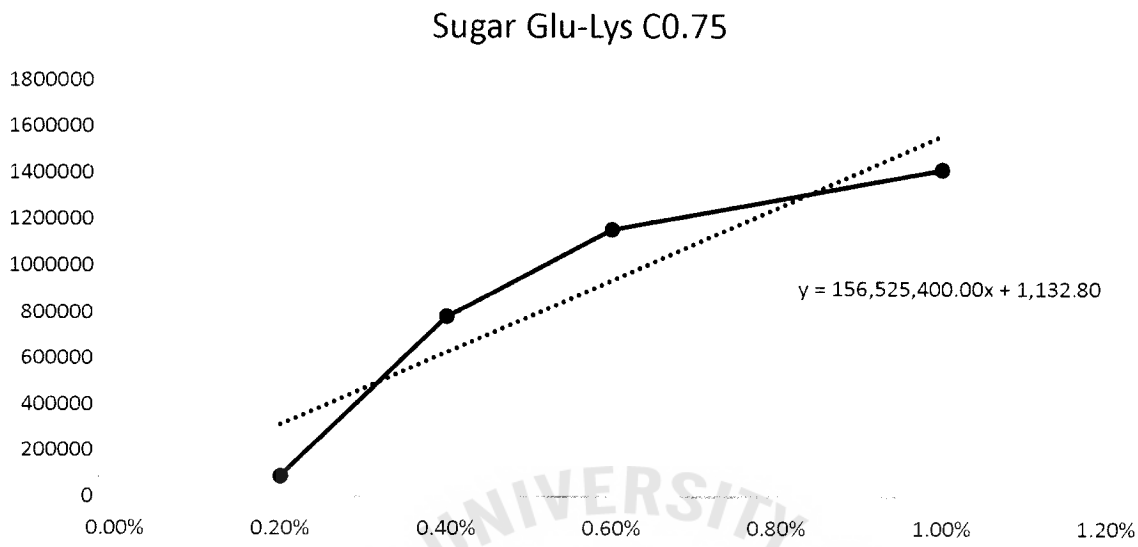
Fructose-Lysine L1.0



Sugar (%)	Area
0.2	347741
0.4	624408
0.6	1004191
1	1321145

Y=	122,672,642.86x + 149,671.71
Slope	122,672,642.86
C	149,671.71

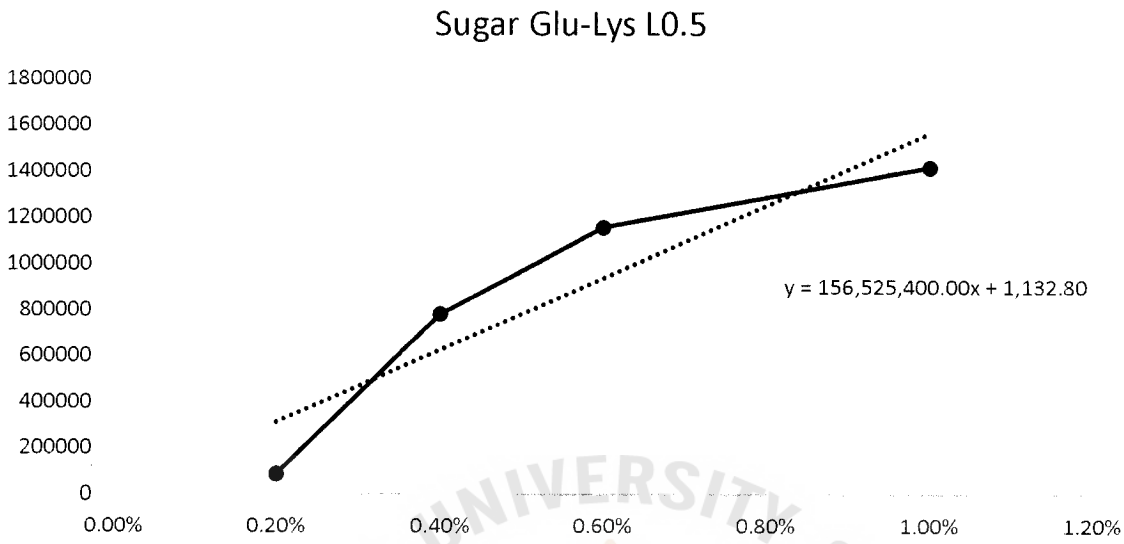
Glucose-Lysine C0.75



Sugar (%)	Area
0.2	88085
0.4	783844
0.6	1157568
1	1418593

Y=	156,525,400x + 1,132.80
Slope	156,525,400
C	1,132.80

Glucose-Lysine L0.5



Sugar (%)	Area
0.2	88085
0.4	783844
0.6	1157568
1	1418593

Y=	156,525,400x + 1,132.80
Slope	156,525,400
C	1,132.80

Best Conditions, Sugar Content Calculations

Y=mx+C , where Y= area, m= slope
HMF= ((area-C)/m)/20unit, unit=mg/ml

Sugar, Fructose- Glycine C1.0

Slope (m) = 123,963,185.71 C= 148,387.23

Hour	Area	Absolute (x)	mg/ml	µg/ml	mean	SD	mean±SD
1	1468569	0.0106	0.0005	0.5325	0.5149	0.0397	0.4751
1	1288521	0.0092	0.0005	0.4599			
1	1517461	0.0110	0.0006	0.5522			
2	1623471	0.0119	0.0006	0.5950	0.6300	0.0058	0.6242
2	1652248	0.0121	0.0006	0.6066			
2	1855253	0.0138	0.0007	0.6885			
3	2185185	0.0164	0.0008	0.8215	0.8875	0.0484	0.8392
3	2469188	0.0187	0.0009	0.9361			
3	2391963	0.0181	0.0009	0.9049			
4	2442698	0.0185	0.0009	0.9254	0.8872	0.0524	0.8348
4	2436696	0.0185	0.0009	0.9230			
4	2164255	0.0163	0.0008	0.8131			
5	1561680	0.0114	0.0006	0.5700	0.5409	0.0218	0.5192
5	1474568	0.0107	0.0005	0.5349			
5	1432114	0.0104	0.0005	0.5178			
6	2489071	0.0189	0.0009	0.9441	0.9046	0.0604	0.8442
6	2504930	0.0190	0.0010	0.9505			
6	2179465	0.0164	0.0008	0.8192			
7	2126630	0.0160	0.0008	0.7979	0.7572	0.0288	0.7285
7	1974477	0.0147	0.0007	0.7365			
7	1976308	0.0147	0.0007	0.7373			

Sugar, Fructose- Glycine L0.75

Slope (m) = 123,963,185.71 C= 148,387.23

Hour	Area	Absolute (x)	mg/ml	µg/ml	mean	SD	mean±SD
1	1137366	0.0080	0.0004	0.3989	0.5759	0.1256	0.4503
1	1828280	0.0136	0.0007	0.6776			
1	1763102	0.0130	0.0007	0.6513			
2	1627605	0.0119	0.0006	0.5966	0.5256	0.0164	0.5092
2	1546112	0.0113	0.0006	0.5638			
2	1180856	0.0083	0.0004	0.4164			
3	1442145	0.0104	0.0005	0.5218	0.5667	0.0318	0.5349
3	1603663	0.0117	0.0006	0.5870			
3	1614368	0.0118	0.0006	0.5913			
4	1525832	0.0111	0.0006	0.5556	0.5708	0.0234	0.5474
4	1519194	0.0111	0.0006	0.5529			
4	1645468	0.0121	0.0006	0.6038			
5	1504907	0.0109	0.0005	0.5471	0.5117	0.0329	0.4789
5	1438038	0.0104	0.0005	0.5202			
5	1308486	0.0094	0.0005	0.4679			
6	1305245	0.0093	0.0005	0.4666	0.4602	0.0137	0.4465
6	1320482	0.0095	0.0005	0.4728			
6	1242237	0.0088	0.0004	0.4412			
7	1213735	0.0086	0.0004	0.4297	0.4123	0.0527	0.3596
7	1304938	0.0093	0.0005	0.4665			
7	993448	0.0068	0.0003	0.3409			

Sugar, Glucose- Glycine C0.5

Slope (m) = 146,384,871.43 C= - 1,701.54

Hour	Area	Absolute (x)	mg/ml	µg/ml	mean	SD	mean±SD
1	947640	0.0065	0.0003	0.3243	0.2938	0.0381	0.2556
1	926347	0.0063	0.0003	0.3170			
1	700987	0.0048	0.0002	0.2400			
2	802977	0.0055	0.0003	0.2749	0.1701	0.0777	0.0924
2	428232	0.0029	0.0001	0.1469			
2	258066	0.0018	0.0001	0.0887			
3	738259	0.0051	0.0003	0.2527	0.1944	0.0544	0.1400
3	609403	0.0042	0.0002	0.2087			
3	354949	0.0024	0.0001	0.1218			
4	542067	0.0037	0.0002	0.1857	0.1620	0.0335	0.1285
4	333899	0.0023	0.0001	0.1146			
4	541853	0.0037	0.0002	0.1857			
5	356458	0.0024	0.0001	0.1223	0.2048	0.0592	0.1455
5	680653	0.0047	0.0002	0.2331			
5	756301	0.0052	0.0003	0.2589			
6	761459	0.0052	0.0003	0.2607	0.2637	0.0117	0.2520
6	733495	0.0050	0.0003	0.2511			
6	815946	0.0056	0.0003	0.2793			
7	859541	0.0059	0.0003	0.2942	0.1943	0.0708	0.1235
7	439959	0.0030	0.0002	0.1509			
7	402030	0.0028	0.0001	0.1379			

Sugar, Fructose- Lysine C1.0

Slope (m) = 202,842,457.14 C= 259,720.49

Hour	Area	Absolute (x)	mg/ml	µg/ml	mean	SD	mean±SD
1	1541391	0.0009	0.0000	0.0464	0.0806	0.0244	0.0562
1	1877013	0.0020	0.0001	0.1016			
1	1829325	0.0019	0.0001	0.0938			
2	1899784	0.0021	0.0001	0.1054	0.1303	0.0332	0.0971
2	2302448	0.0034	0.0002	0.1717			
2	1950632	0.0023	0.0001	0.1138			
3	2232495	0.0032	0.0002	0.1602	0.1310	0.0210	0.1100
3	1996969	0.0024	0.0001	0.1214			
3	1936902	0.0022	0.0001	0.1115			
4	1673489	0.0014	0.0001	0.0681	0.0412	0.0193	0.0219
4	1449814	0.0006	0.0000	0.0313			
4	1406485	0.0005	0.0000	0.0242			
5	1541265	0.0009	0.0000	0.0464	0.0526	0.0142	0.0383
5	1496907	0.0008	0.0000	0.0391			
5	1698547	0.0014	0.0001	0.0723			
6	1856635	0.0020	0.0001	0.0983	0.0853	0.0104	0.0748
6	1701898	0.0015	0.0001	0.0728			
6	1773831	0.0017	0.0001	0.0847			
7	1370159	0.0004	0.0000	0.0182	0.0001	0.0218	-0.0217
7	1336907	0.0003	0.0000	0.0127			
7	1073818	-0.0006	0.0000	-0.0306			

Sugar, Fructose- Lysine C1.0

Slope (m) = 122,672,642.86x C= 149,671.71

Hour	Area	Absolute (x)	mg/ml	µg/ml	mean	SD	mean±SD
1	1007745	0.0070	0.0003	0.3497	0.4133	0.0527	0.3606
1	1324394	0.0096	0.0005	0.4788			
1	1159242	0.0082	0.0004	0.4115			
2	1130746	0.0080	0.0004	0.3999	0.3605	0.0394	0.3211
2	937524	0.0064	0.0003	0.3211			
2							
3	1261381	0.0091	0.0005	0.4531	0.5131	0.0424	0.4707
3	1480142	0.0108	0.0005	0.5423			
3	1484027	0.0109	0.0005	0.5439			
4	1442209	0.0105	0.0005	0.5268	0.4918	0.0279	0.4639
4	1274521	0.0092	0.0005	0.4585			
4	1352014	0.0098	0.0005	0.4901			
5	1334289	0.0097	0.0005	0.4828	0.4783	0.0363	0.4420
5	1426131	0.0104	0.0005	0.5203			
5	1208829	0.0086	0.0004	0.4317			
6	1472858	0.0108	0.0005	0.5393	0.5070	0.0234	0.4836
6	1338884	0.0097	0.0005	0.4847			
6	1368742	0.0099	0.0005	0.4969			
7	1392934	0.0101	0.0005	0.5067	0.5200	0.0243	0.4958
7	1509047	0.0111	0.0006	0.5541			
7	1374718	0.0100	0.0005	0.4993			

Sugar, Fructose- Glycine C1.0

Slope (m) = 123,963,185.71 C= 148,387.23

Hour	Area	Absolute (x)	mg/ml	µg/ml	mean	SD	mean±SD
1	1468569	0.0106	0.0005	0.5325	0.5149	0.0397	0.4751
1	1288521	0.0092	0.0005	0.4599			
1	1517461	0.0110	0.0006	0.5522			
2	1623471	0.0119	0.0006	0.5950	0.6300	0.0058	0.6242
2	1652248	0.0121	0.0006	0.6066			
2	1855253	0.0138	0.0007	0.6885			
3	2185185	0.0164	0.0008	0.8215	0.8875	0.0484	0.8392
3	2469188	0.0187	0.0009	0.9361			
3	2391963	0.0181	0.0009	0.9049			
4	2442698	0.0185	0.0009	0.9254	0.8872	0.0524	0.8348
4	2436696	0.0185	0.0009	0.9230			
4	2164255	0.0163	0.0008	0.8131			
5	1561680	0.0114	0.0006	0.5700	0.5409	0.0218	0.5192
5	1474568	0.0107	0.0005	0.5349			
5	1432114	0.0104	0.0005	0.5178			
6	2489071	0.0189	0.0009	0.9441	0.9046	0.0604	0.8442
6	2504930	0.0190	0.0010	0.9505			
6	2179465	0.0164	0.0008	0.8192			
7	2126630	0.0160	0.0008	0.7979	0.7572	0.0288	0.7285
7	1974477	0.0147	0.0007	0.7365			
7	1976308	0.0147	0.0007	0.7373			

Sugar, Fructose- Glycine C1.0

Slope (m) = 123,963,185.71 C= 148,387.23

Hour	Area	Absolute (x)	mg/ml	µg/ml	mean	SD	mean±SD
1	1137366	0.0080	0.0004	0.3989	0.5759	0.1256	0.4503
1	1828280	0.0136	0.0007	0.6776			
1	1763102	0.0130	0.0007	0.6513			
2	1627605	0.0119	0.0006	0.5966	0.5256	0.0164	0.5092
2	1546112	0.0113	0.0006	0.5638			
2	1180856	0.0083	0.0004	0.4164			
3	1442145	0.0104	0.0005	0.5218	0.5667	0.0318	0.5349
3	1603663	0.0117	0.0006	0.5870			
3	1614368	0.0118	0.0006	0.5913			
4	1525832	0.0111	0.0006	0.5556	0.5708	0.0234	0.5474
4	1519194	0.0111	0.0006	0.5529			
4	1645468	0.0121	0.0006	0.6038			
5	1504907	0.0109	0.0005	0.5471	0.5117	0.0329	0.4789
5	1438038	0.0104	0.0005	0.5202			
5	1308486	0.0094	0.0005	0.4679			
6	1305245	0.0093	0.0005	0.4666	0.4602	0.0137	0.4465
6	1320482	0.0095	0.0005	0.4728			
6	1242237	0.0088	0.0004	0.4412			
7	1213735	0.0086	0.0004	0.4297	0.4123	0.0527	0.3596
7	1304938	0.0093	0.0005	0.4665			
7	993448	0.0068	0.0003	0.3409			

BIOGRAPHY

Name:	Ms. Shriya Gorowara
Date of Birth:	6th October 1994
Place of Birth	Bangkok, Thailand
Institutions Attended:	Assumption University of Thailand, Faculty of Biotechnology 2010-2014
	Modern International School of Bangkok 1998-2010