# ANTIGLYCATION EFFECTS OF EXTRACTS FROM SIX POMELO CULTIVARS

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A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science Program in Food and Nutrition

Department of Nutrition and Dietetics

Faculty of Allied Health Sciences

Chulalongkorn University

Academic Year 2011

# ฤทธิ์ต้านไกลเคชั่นของส้มโอ 6 สายพันธุ์

นางสาวณัฐรินทร์ แสงปราสาท

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตร์มหาบัณฑิต
สาขาวิชาอาหารและโภชนาการ ภาควิชาโภชนาการและการกำหนดอาหาร
คณะสหเวชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2554

ณัฐรินทร์ แสงปราสาท : ฤทธิ์ต้านไกลเคชั่นของส้มโอ 6 สายพันธุ์. (ANTIGLYCATION EFFECTS OF EXTRACTS FROM SIX POMELO CULTIVARS) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: รศ.ดร.สิริชัย อดิศักดิ์วัฒนา, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม อ.ดร. สถาพร งามอุโฆษ, 141 หน้า.

ภาวะระดับน้ำตาลในเลือดสูงทำให้เกิดกระบวนการไกลเคชั่นซึ่งนำไปสู่การสร้างผลิตภัณฑ์แอดวานซ์ ไกลเคชั่น เอ็น โปรดักส์(advanced glycation end-products) ซึ่งเป็นผลิตภัณฑ์สำคัญที่ทำให้เกิดภาวะแทรกซ้อนของโรคเบาหวาน เช่นความผิดปกติทางไต (diabetic nephropathy) และความผิดปกติทางเส้นประสาท(diabetic neuropathy) ในปัจจุบันมีการศึกษาสารจากธรรมชาติจากพืชและผลไม้ที่มีคุณสมบัติในการต้านปฏิกิริยาไกลเคชั่น เพื่อป้องกันและรักษาโรคแทรกซ้อนที่จะเกิดขึ้นเบาหวาน ส้มโอเป็นผลไม้ที่มีความนิยมในประเทศไทยซึ่งมีการค้นพบว่าสารฟลาโวนอยด์ในส้มโอมีประโยชน์ต่อสุขภาพ วัตถุประสงค์ของการวิจัยนี้เพื่อศึกษาสารฟลาโวนอยด์ในส้มโอทั้งหกสายพันธ์ได้แก่ ท่าข่อย ขาวน้ำผึ้ง ขาวแตงกวา ทองดี ทับทิมสยามและขาวใหญ่ และศึกษาถึงความสามารถของสารสกัดจากเนื้อส้มโอทั้งหกสายพันธุ์นี้ในการยับยั้งกระบวนการไกลเคชั่นของโปรตีนอัลบูมินจากวัว (bovine serum albumin) ที่ถูกเหนี่ยวนำให้เกิดปฏิกริยาไกลเคชั่นโดยน้ำตาลฟรุกโตส ผลการทดลองพบว่าส้มโอทั้งหกสายพันธุ์ประกอบไปด้วยสารฟลาโวนอยด์ได้แก่ นารินจิน (naringin), เฮสพิริดิน (hesperidin), นีโอเฮสพิริดิน (neohesperidin), นีโอเฮสพิริดิน ไดไฮโดรชาลโคน (neohesperidin dihydrochalcone), นารินจีนิน (naringenin) และ เฮสพิรทิน (hesperitin) สารสกัดส้มโอทั้งหกสายพันธุ์ที่ความเข้มข้น 0.25 – 2.00 มิลลิกรัมต่อมิลลิตร สามารถยับยั้งการเกิดผลิตภัณฑ์แอดวานซ์ ไกลเคชั่น เอ็น โปรดักส์ และ เอ็นเอปซิลอน-คาร์บอกซีเม็ททิลไลซีน ($N^{\varepsilon}$-(carboxymethyl)lysine) ตามค่าความเข้มข้นที่เพิ่มขึ้นของสารสกัดจากเนื้อส้มโอ ส้มโอทั้งหกสายพันธุ์สามารถลดปริมาณสารฟรุกโตซามีนซึ่งสัมพันธ์กับการลดลงของปริมาณผลิตภัณฑ์แอดวานซ์ ไกลเคชั่น เอ็น โปรดักส์ สารสกัดจากเนื้อส้มโอช่วยยับยั้งปฏิกิริยาออกซิเดชั่นโดยสามารถเพิ่มปริมาณหมู่ไธออลในโปรตีนอัลบูมินและลดระดับโปรตีนคาร์บอนิล (carbonyl) ซึ่งแสดงว่าสารสกัดส้มโอสามารถป้องกันการเกิดโปรตีนออกซิเดชั่นในโปรตีนอัลบูมินได้ นอกจากนี้สารสกัดส้มโอสามารถลดปริมาณโครงสร้างอะไมลอยด์ ครอสลิ้งค์ เบต้า (amyloid cross linked β-structures) ในโปรตีนอัลบูมิน ซึ่งแสดงให้เห็นถึงการลดความเสี่ยงของการเกิดพยาธิสภาพต่างๆ ผลจากการทดลองนี้ยังแสดงให้เห็นว่าสารสกัดจากเนื้อส้มโอทั้งหกสายพันธุ์มีความเป็นไปได้ที่จะมาประยุกต์ใช้เป็นผลิตภัณฑ์เสริมอาหารเพื่อป้องกันการเกิดโรคแทรกซ้อนจากโรคเบาหวานที่เกิดขึ้นจากผลิตภัณฑ์แอวานซ์ ไกลเคชั่น เอ็น โปรดักส์

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| ภาควิชาโภชนาการและการกำนดอาหาร | ลายมือชื่อนิสิต..... |
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| ปีการศึกษา 2554 | ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม..... |

## 5377302037 : MAJOR FOOD AND NUTRTION

KEYWORDS : POMELO / GLYCATION / FLAVONOIDS / DIABETES MELLITUS / ANTI-GLYCATION

NATARIN CAENGPRASATH : ANTIGLYCATION EFFECTS OF EXTRACTS FROM SIX POMELO CULTIVARS. ADVISOR: ASSOC. PROF. SIRICHAI ADISAKWATTANA, Ph.D., CO-ADVISOR : SATHAPORN NGAMUKOTE, Ph.D., 141 pp.

Chronic hyperglycemia promotes the formation of non-enzymatic protein glycation, leading to the production of advanced glycation end-products (AGEs), which play a significant role in the development of complications such as diabetic nephropathy and neuropathy. Immense efforts have been devoted into finding effective antiglycation compounds from dietary plants and fruits for prevention and the treatment of diabetic complications. Pomelo (*Citrus grandis (L.) Osbeck*), one of the Thailand's largest citrus fruits, contains of many flavonoids that have shown to beneficial effects on human health. The objective of this study was to quantify of flavonoids in six pomelo cultivars (Tar Koi, Kao Nam Pueng, Kao Tanggwa, Thong Dee, Tubtim Siam and Kao Yai) and to determine the preventive effect of the six pomelo cultivars against a fructose induced non enzymatic glycation assay of bovine serum albumin (BSA). The results revealed that the six pomelo cultivars contained of naringin, hesperidin, neohesperidin, neohesperidin dihyrodchalcone, naringenin and hesperitin. It was found that the six pomelo cultivar extracts (0.25 – 2.00 mg/ml) significantly inhibited the overall formation of AGEs and $N^{\varepsilon}$-(carboxymethyl)lysine (CML) in a concentration-dependent manner. In addition, the six pomelo cultivar extracts decreased the formation of fructosamine, which is directly associated to the reduction of the AGE formation. The pomelo cultivar extracts were also able to suppress the formation of protein oxidation through its ability to increase levels of thiol groups and reduce the amount of protein carbonyl formation, indicating of protection of protein oxidation in BSA. Moreover, the pomleo cultivar extracts showed abilities in suppressing formation of amyloid cross linked β-structures of BSA, suggesting of a reduced risk in developing debilitating degenerative diseases. These results reveal that the six pomelo cultivar extracts may be a useful dietary supplement for preventing AGE-mediated diabetic complications.

Department : Nutrition and Dietetics ..... Student's Signature .....

Field of Study : Food and Nutrition ..... Advisor's Signature .....

Academic Year : 2011 ..... Co-advisor's Signature .....

# Acknowledgements

First and foremost, I would like to express my sincere gratitude to my advisor Assoc. Prof. Dr. Sirichai Adisakwattana for the continuous support of my Master's study and research, for his patience, motivation, enthusiasm and unsurpassed knowledge. His guidance not only helped me throughout the whole time of this research and writing of this thesis but also thought out my course of study.

At the side of my advisor, I would like to thank my co-advisor Dr. Sathaporn Ngamukote for all her kind assistance, suggestion and continuous support towards my research. This thesis would not have been complete without her help, for which I am extremely grateful for.

My sincere thanks also goes to the rest of my thesis committee: Associate Professor Dr. Winai Dahlan, Dr. Sathaporn Ngamukote, Dr. Kittana Mäykenen and Dr. Chatrapa Hudthagosl for their constructive guidance and suggestions. Without it, this thesis would not have been this successful.

Among my fellow postgraduate students in the faculty of Allied Health Sciences, I would like to thank you for all the warm hospitality and constant support. I also would like to thank you for all their kind assistance and for accommodating to all my queries.

I would like to thank my former advisor Dr. Christopher Smith, whose good advice, generosity, support and friendship, for which has been invaluable on both an academic and a personal level.

Last but not least, I thank my family members for all the continuous support and great patience. Most of all, I would like thank for their unequivocal encouragement to pursue this degree. Without my family, I wouldn't have been able to achieve what I have accomplished today.

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# Chapter I

# Introduction

## Background and significant of the study

Diabetes mellitus is characterized by hyperglycemia subsequent with defective insulin production (Type 1 Diabetes), insulin resistance (Type 2 Diabetes) or both types combined. With the occurrence of diabetes mellitus rapidly increasing every year, it is widely accepted that not only does it create an economic burden to the world but also many social ones. It has been reported that the incidence of diabetes mellitus is increasing every year that affects about 6% of the world's population (Rudkowska, 2009). It is predicted that the incidence of diabetes will be double by the year 2030 due mainly to sedentary lifestyle and obesity (Wild et al., 2004). Like any other disease, diabetes mellitus brings upon several adverse complications primarily due to hyperglycemia, which can lead to morbidity and mortality. Such complications can cause acute metabolic changes in lipid and protein metabolism and more dire chronic irreversible complications (Brownlee, 2005). These diabetes specific chronic irreversible complications include microvascular complications (retinopathy, nephropathy and neuropathy) and macrovascular complications (atherosclerosis) (Fowler, 2008). From the many mechanisms that cause the onset and progression of diabetes complications, one of the main primary mechanisms responsible is glycation.

Glycation is a non-enzymatic reaction that occurs between a carbonyl group of a sugar and an amino group of an amino acid, leading to the formation of N-substituted glycosylamine and water (Lapolla et al., 2005). This non-enzymatic reaction causes a rearrangement of the reducing sugar and proteins, providing the basis for more reactions to

occur such as Schiff base condensation, and Amadori reactions (Peyroux et al., 2006). These reactions then lead to advanced glycation end-products (AGEs), particularly when the Amadori reaction causes its product to be oxidized by a transition metal catalyst (Wautier et al,. 2001). The formation of advanced glycation end-products not only leads to acute and irreversible chronic complications but also causes the formation of reactive oxygen species (ROS)-induced oxidation, and beta amyloid protein formation (Miyata et al., 1993). ROS-induced oxidative stress, and glycation or glycoxidation can alter protein conformation, alter enzyme activity, modify protein half life, and alter immunogenicity, cross-linking and denaturation behavior of proteins. Moreover, the interactions of advanced glycation end-product receptors (RAGEs) with AGEs can cause further oxidative stress and activation of the nuclear factor kB (NF-κB). As a consequence, the generation of pro-inflammatory adhesion molecules results in increased vascular permeability (Ahmad et al., 2006).

Due to glycation being a primary mechanism responsible for adverse complications in diabetes mellitus, much effort and attention has been devoted to develop therapeutic strategies targeting this reaction and to alleviate its complications. A common strategy is to design antiglycation compounds, which act by blocking carbonyl groups on reducing sugars, formation of Amadori products, and 3-deoxyglucosones, which then inhibit the formation of AGEs (Ahmad et al., 2006). Drugs, for instance aminoguanidine, act to cleave AGE cross-links and block receptors of AGEs (Cameron et al., 2005). Furthermore, antioxidants and chelators can also be used for prevention of glycoxidation, and to remove transition metals such as iron and copper. Although antiglycation compounds having multiple modes of action to inhibit AGE formation are under development, most of these are still at the early levels of clinical and experimental evaluation. As an alternative to these, natural products such as nutrients, vitamins and medicinal plant extracts have also shown potential to inhibit overall AGE formation, and these may offer an alternative to expensive synthetic therapeutics. Nutrients and vitamins such as ascorbic acid, folic acid, zinc (Tupe

et al., 2010a), vitamin E, vitamin B6 and vitamin B3 (Vinson et al., 1996a) have shown to be capable of inhibiting AGE formation. Additionally, a variety of plant extracts including garcinol (Yamaguchi et al., 2000), garlic extract (Ahmad et al., 2006), tomato extract (Kiho et al., 2004), and *Camellia sinesis* extract (Nakagawa et al., 2002) have shown promising results in inhibiting glycation. Hence, the potential of natural products has attracted vast amounts of interest in the prevention of diabetic complications.

Pomelo, a native citrus fruit of Southeast Asia and a cash crop to Thailand has been shown to hold an array of flavonoids including hesperidin, neohesperidin, kaempferol, rutin, apigenin, quercetin, narirutin and naringin (Theppakorn et al., 2009). Moreover, this citrus fruit also provides an ample supply of vitamin C, folic acid, potassium and pectin (Benavente-García et al., 1997). Pomelo flavonoids have also shown potential health benefits, exerting antiviral, anti-allergic, anti-platelet, anti-inflammatory, antitumor, antioxidant and even antiglycation activity (Benavente-García et al., 1997; Vinson et al,. 1996). However, studies related to the antiglycation properties of pomelo flavonoids are limited.

## Purpose of this study

1. To quantify the relative amounts of flavonoids present in six different pomelo cultivars

2. To evaluate the antiglycation effects of extracts from six cultivars of pomelo against a fructose induced non enzymatic glycation assay of bovine serum albumin (BSA) and assess oxidation-dependent damage to BSA mediated by fructose.

## Research hypothesis and rationale

The hypothesis of this study is that the pomelo cultivars will contain a number of flavonoids, those similar to citrus flavonoids and that each pomelo cultivar and its flavonoids constituents will inhibit overall formation of AGEs. The hypothesis was be tested by studying the effect of each pomelo culitvar and its flavonoid constituents against bovine serum albumin (BSA) in a fructose induced non enzymatic glycation and oxidation-dependent damages to BSA mediated by fructose.

## Expected benefits of the study

Thailand is a developing country with a large portion of the population having low income, and fewer socioeconomic benefits in comparison to Western countries. Thus, necessities such as widely distributed medical attention, and accessible and affordable therapeutic strategies especially to degenerative and diet related diseases like diabetes mellitus are insufficient and cost-inhibitive to many people. The pomelo extract can be developed it into a nutraceutical food that is cost effective and widely accessible may be merited. The extracts can be applied as a component of medical foods and or an alternative to medication. Development and commercialization of the extracts not only hold great health benefits but also economical benefits and that it is advantageous to indigenous communities as well as to the health of the general population.

# Chapter II

# Review of Literature

## 1. Diabetes Mellitus

Diabetes mellitus is a group of metabolic disorders which result from a disordered glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism (World Health Organization, 1999). A disordered glucose homeostasis is primarily due to defects in insulin secretion, action, or both, or in the biological effectiveness of insulin. The disorder is characterized by chronic hyperglycemia and can cause long term damage, dysfunction and failure to various organs (American Diabetes Association, 2008)

### 1.1 Type and etiology of Diabetes mellitus

Chronic hyperglycemia can be classified into two categories which group disorders, defects or processes that cause a disordered glucose homeostasis. The two categories are termed type I, or immune mediated-diabetes, and type II diabetes.

#### 1.1.1 Type 1 Diabetes

Type 1 diabetes occurs *via* beta-cell destruction of the pancreas which causes it to produce insufficient levels of insulin (Bloomgarden, 2009). Hence, an absolute insulin deficiency results and exogenous insulin is required. Type 1 can be characterized by the presence of anti glutamic acid decarboxylase (anti-GAD), islet cell (ICA) or insulin antibodies (IA-2) which identify the autoimmune processes that induce beta-cell destruction (Cnop et al., 2005).

The autoimmune destruction of the pancreatic beta cells is caused by an inflammatory reaction that transpires through the invasion of insulitis, or mononuclear cells, to the islets of the pancreas (Kloppel et al., 1985). The insulitis come into direct contact with the islet cells through the means of activated macrophages and T-cells, resulting in their exposure to secreted intermediaries of these cells, which are composed of cytokines, nitric oxide (NO), and oxygen free radicals. As a result, lengthened exposure to such intermediaries causes insulitis function impairment and beta-cell death (Eizirik et al., 2001).

### 1.1.2 Type 2 Diabetes

Type 2 diabetes, one of the more common forms of diabetes is also defined by progressive beta-cell failure; however the condition is more variable than type 1 diabetes. The severity of type 2 diabetes is variable, depending level of insulin resistance in the patients (Dickinson et al., 2002). Insulin resistances are evoked by loss of acute glucose-induced insulin secretion (GIIS) which then leads to insulin action and secretion disorders (Cnop et al., 2005). Precise reasons for onset of the disorder are unknown; however hereditary factors and diet are believed to be factors influencing its development. Thus, chronically elevated blood glucose and fatty acid levels, due to obesity and glucose intolerance, a loss of expression pattern of genes required for GIIS occurs (Tokuyama et al., 1995) (Weir et al., 2001). As a result, hyperglycemia and hyperlipidemia is exacerbated and further beta-cell differentiation occurs. Moreover, as in type 1 diabetes, inflammation mechanisms are believed to also play a role in the disorder.

## 2. Complications of Diabetes Mellitus

Hyperglycemia can be treated through vast array of lifestyle and pharmaceutical interventions aimed at preventing and controlling hyperglycemia. However, if hyperglycemia is lacked of proper care progression of many complications can occur, varying from short-term to long term irreversible complications.

### 2.1 Microvascular

Microvascular complications of diabetes occur when there is an angiopathy affecting small blood vessels causing them to become damaged, leading to diabetic retinopathy, diabetic nephropathy and diabetic neuropathy (Ahmed, 2005). Diabetic retinopathy, one of the most common microvascular complication diabetes occurs when there are small hemorrhages in the middle layers of the retina and if proliferative can cause blindness. In diabetic nephropathy, nephrons become thicken and become scarred over time of which prolong scarring can lead to renal failure (Saito et al., 2005). Diabetic neuropathy, is defined as the presence of symptoms and/or occurences of peripheral nerve dysfunction in patients (Saraswat et al., 2009). Nonetheless, severity of each complication is in relation to both the magnitude and duration of hyperglycemia.

### 2.2 Macrovascular

Macrovascular complications in diabetes are underlined by the process of atherosclerosis, where arterial walls are narrowed and thicken progressively over time. Narrowing and thickening of the arterial walls is caused by the buildup of plaque due to endothelial injury and inflammation. Inflammation is transpired due to the infiltrate of macrophage white bloods cells and which attracts oxidized lipids to further accumulate

the arterial walls (Hartog et al., 2007). Consequently, the T-lymphocytes induce smooth muscle proliferation and further accumulation of collagen (Goldin et al., 2006). As a result, a formation of rich-lipid atherosclerotic lesion and with rupture acute vascular infarction occurs. Of which in proportionate to hyperglycemia, accumulation and narrowing of the walls is exacerbated (Vlassara et al., 2002).

### 2.2.1 Molecular mechanisms of complications

Hyperglycemia is primarily the one of the main causes for complications and its progression. Hyperglycemia induces the many processes that underline such complications, consisting of the acceleration of the polyol pathway, activation of protein kinase C, production of reactive oxygen species and accumulation of advanced glycation end-products (Kang et al., 1999).

#### 2.2.1.1 Sorbitol-aldose reductase (Polyol Pathwayl)

With hyperglycemia, the polyol pathway is activated. At high glucose concentrations the glucose is reduced by the enzyme aldose reductase and its co-factor adenine dinucleotide phosphate to a sorbitol and the sorbitol is then further oxidized by the sorbitol dehydrogenase (SDH) to fructose (Chung et al., 2003).

Hyperglycemia induced sorbitol accumulation, especially in cells that are insulin independent like the retina; kidney and nervous tissues can lead to an excessive usage of the NADPH causing an imbalance of this coenzyme for other cellular energy metabolism processes (Niedowicz et al., 2005). At normal state NADPH promotes the production of nitric oxide and glutathione, however with a shift of its availability it can cause deficiency nitric oxide and glutathione

will hence, be insufficient (Ellis, 2007). Insufficient glutathione can lead to hemolysis derived from oxidative stress. A deficiency of nitric oxide can lead to a vasoconstriction and an overall poor blood supply (Sevier et al., 2002).

Disproportionate activation of the polyol pathway can increases the concentrations of intracellular and extracellular sorbitol. The increased levels of reactive oxygen species are related to the decreased concentrations of nitric oxide and glutathione. Consequently an imbalance of these factors causes overall cell damage (Chung et al., 2003).

2.2.1.2 Activation of protein kinase C

Protein kinase C (PKC) is a family of enzymes that participate in regulating the function of other proteins through means of phosphorylation. However, due to high concentrations of glucose, PKC activity can be poorly regulated and is overly enhanced (Pfeiffer, 1996). When PKC is activated by intracellular hyperglycemia, the synthesis of diacylglycerol is increased, causing various vascular abnormalities that effect the modulation of enzymatic activities which are normally regulated by PKC (Turttle et al., 2009; Mamputu et al., 2002). Accordingly, alterations to the retinal and renal blood flow, contractility, permeability and high levels of cytokines production and of extracellular matrix occur. Moreover,cell proliferation and angiogenesis in vascular cells also take place .

2.2.1.3 Production of reactive oxygen species

Due to persistent hyperglycemia together with the activation of many processes that amplify the cells integrity, production of free radicals and

especially reactive oxygen species (ROS) increase (Hunt et al., 1993). Free radicals and ROS are generated as a result of metabolism of oxygen, however at any state of stress an imbalance of the production and manifestation takes place (Loske et al., 1998). The imbalanced level leads to multiple damages to cellular proteins, membrane lipids and nucleic acids and cell death.

2.2.1.4 Non-enzymatic glycosylation

At high concentrations of glucose the bonding of a protein molecule with a sugar molecule occurs without the regulation of an enzyme (Goh et al., 2008). Like other mechanisms induced by high concentrations functions of biomolecules are impaired. Prolonged and further happening of the non-enzymatic glycosylation eventually leads to the formation of advanced glycation end – products which cause further damage (Araki et al., 2004).

3. Advanced Glycation End-Products

From the many contributions leading to the complications of diabetes mellitus, the formation and subsequent effects of advanced glycation end-products (AGEs) are predicted to be the largest contributor (Rojas et al., 2004). AGEs are also believed to cause further complications such as atherosclerosis and Alzheimer's disease, and assist in premature ageing (Fowler, 2008)

3.1 Formation of AGEs

The formation of AGEs arises from a series of various chemical reactions, including condensations, rearrangements, fragmentations, and oxidative structural modifications. A non-enzymatic reaction between a reducing sugar and the amino

groups of proteins (Goh et al., 2008), a Schiff base reaction, generates intermediates which undergo further cascade transformations *via* Amadori, and Malliard reactions leading to the formation of AGEs (Peyroux et al., 2006), figure 1.

The first stage of AGEs formation begins with formation of a Schiff base. A Schiff base is formed when the aldehyde group of a reducing sugar molecule reacts with the amino group of a lysine molecule, producing a double bond between the carbon atom of the aldehyde and the nitrogen atom of the lysine (Yim et al., 2001). The Schiff base then undergoes an Amadori rearrangement, where a hydrogen atom from the hydroxyl group adjacent to the carbon-nitrogen double bond shifts to the nitrogen center, resulting in a carbonyl group (Isbell et al., 1958).

The Amadori product undergoes oxidation reactions, forming multiple mediators that are believed to be precursors to the formation of AGEs. These mediators include α-dicarbonyl species such as *a*-oxoaldehydes, methylglyoxal, glyoxal and the formation of the highly reactive 3-deoxyglucosone, which results from the degradation of the Amadori product (Sharma et al., 2002).

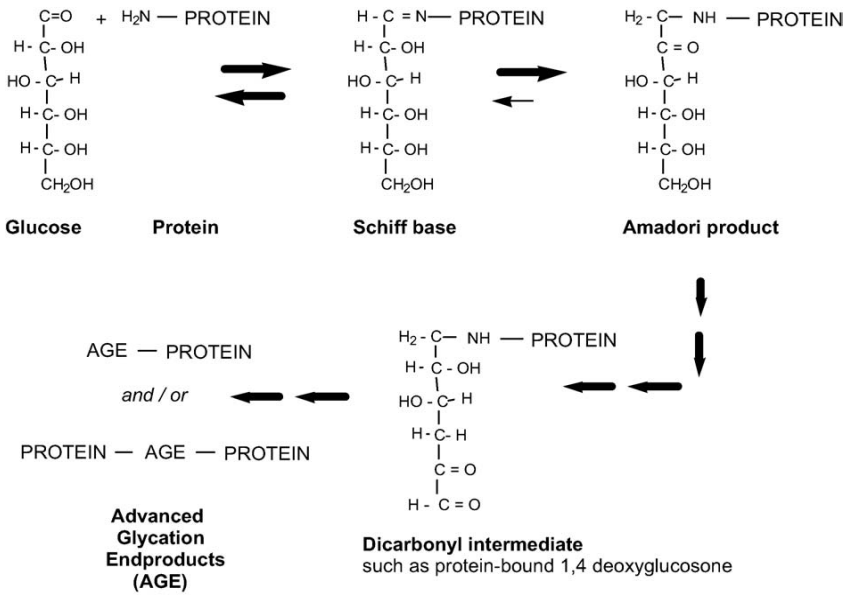



Fig. 1 Pathway of protein glycation by glucose leading to AGEs (Peyroux et al., 2006).

### 3.2 Glycoxidation

As a result of diabetes mellitus, hyperglycemia is accompanied by an increased production of free radicals or impaired antioxidant defenses. However, oxidative stress not only results from hyperglycemia but also through processes pre-, and post-AGEs formation, such as non-enzymatic protein glycosylation, auto-oxidation of glucose, impaired glutathione metabolism, alterations in antioxidant enzyme activity and lipid peroxide formation (Moussa, 2008).

The formation of an early glycation product (Ahmed et al., 2002), a ketoamine, is primarily responsible for giving rise to the above processes. This is generated from a Schiff base of a monosaccharide undergoing the Amadori rearrangement. When the monosaccharide is specifically fructose, the ketoamine formed is a *N*-(1-deoxy-D-fructos-1-yl)-amino acid, or fructosamine (Armbruster, 1987; Smith et al., 1992). The formed fructosamine then undergoes complex cascades of dehydration, oxidation, condensation, fragmentation and cyclization reactions (Thome et al., 1996). As a result of these, particularly from oxidation reactions, proteins are oxidatively attacked, leading to the formation of extra carbonyl groups (aldehydes and ketones). These groups can also be further introduced into proteins through secondary reactions of the protein residues (Dalle-Donne et al., 2003). The increase in carbonyl group functionality, and fructosamine formation are two main factors leading to the formation of AGEs (Levine et al., 2000).

Similar to the formation of fructosamine, cross β-amyloid structures can also be formed through the same process. Cross β-amyloid structures are generated through

the irreversible cross-linking of heterogeneous protein aggregates derived from the formation of AGEs (Smith et al., 2000). In combination with oxidation processes, the cross β-amyloid structures generate insoluble β-amyloid plaques and β-sheet fibrils (Vitek et al., 1994). The insoluble amyloid β-plaques and β-sheet fibrils have been shown to promote further oxidative stress in neuronal cells (Olivieri et al., 2001). In addition to oxidative stress from the formation of AGEs fostering the development of cross β-amyloids, reduced levels of oxidized glutathione can also contribute to the occurrence of glycoxidation. Glutathione is an intracellular antioxidant, preventing over-oxidation of proteins by scavenging reactive oxidative intermediates. Thus, in neuronal cultures decreased levels of glutathione in its oxidized form can be an indication of free radical stress and hence glycoxidation (Woltjer et al., 2005).

With increased levels of intracellular oxidative stress, hyperglycemia induced reactive oxygen species can activate a range of inflammation responses which triggers cytokines, such as transcription factor NF-κB (Katsuki et al., 1998). Transcription factor NF-κB in turn enhances the production of nitric oxide, which can lead to disorders, and defects in certain organs (Dickinson et al., 2002).

### 3.3 AGE binding receptors

Glycation reactions result in a large number of potential AGEs. The binding and degradation of these by AGE binding receptors results not only in their removal, but also leads to diabetes related complications. The degradation products of AGEs activates proinflammatory, and prothrombotic pathways (Hudson et al., 2002) resulting in many deleterious effects in diabetic patients.

## 3.4 The types of AGEs Structure

Facilitating the AGEs to react through arrays of mechanisms and pathways are a variety of AGEs adducts with fluorescent and brown color characteristics. These adducts can be structures resulting from formation of protein-protein cross links, or other structures not containing non-cross linked proteins (Ulrich et al., 2001).

### 3.4.1 Fluorescent AGE cross-linked structures

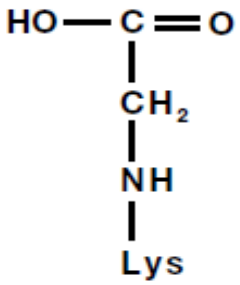
The reaction between the $\alpha$-dicarbonyl intermediate and an amino acid residue during the Schiff's base formation can result in a protein-protein cross-linked structures incorporating fluorescent chromophores (Ulrich et al., 2001).

### 3.4.2 Non-cross linking AGE structures

Aside from cross-linking AGE structures, other structures are also formed which contain no protein cross-links. Non-cross linking AGE structures are also formed initially by the same reaction of a $\alpha$-dicarbonyl intermediate and amino acid residues or with Amadori intermediates, however these structures are formed based on glycoxidation more than the protein-protein cross linked structures and do not have fluorescent chromophoric characteristics (Ulrich et al., 2001). The non-cross linking AGEs result through the oxidative cleavage of Amadori adducts, oxidative cleavage of Schiff base and modification with glyoxal generated by autoxidation of glucose (Nagai et al., 2002).

One of the most commonly found, and most abundant non-cross linked AGE structures in protein tissue is the $N^{\varepsilon}$-(carboxymethyl)lysine (CML) structure (Ikeda et al., 1996), figure 2. The accumulation of CML is often enhanced under

conditions of chronic hyperglycemia and in cases of renal failure (Kislinger et al., 1999), with its presence in vascular tissue leading to tissue damage, and increases in vascular permeability which can heighten the risk of atherosclerosis (Ikeda et al., 1996; Wautier et al., 2003). Additionally, vascular cell adhesion molecules are activated which cause the production of cytokines and tissue expression factors such as transcript nuclear factor alpha (TNF-$\alpha$), which promotes the onset of many degenerative diseases.




CML

Fig 2. The non-crosslinked AGE structure, CML (Antibodies against AGEs-modified protein and AGE receptor)

3.5 The metabolism of AGEs

Resulting from hyperglycemia, the liver is one of the organs that are largely affected. Due to this, the liver is considered the main site of AGE metabolism. The metabolism of AGEs is mediated through the Kupffer cells and the liver endothelial cells, cells which primarily function to remove non physiological and physiological ligands for scavenger receptors (Nagai et al., 2002). Hence, if damaged were to be cause to the liver or liver impairment through, for instance, cirrhosis, a common

complication associated with diabetes mellitus, may cause an elevation of AGEs (Smedsrød et al., 1997)

## 3.6 AGE effects on different organs in the body

Enhanced AGE accumulation can cause the development to many diabetic complications which in turn can lead to the impairment of many vital organs.

### 3.6.1 Vascular and Heart

Enhanced AGE accumulation can lead to the development of cardiac dysfunction through pathways that can cause or accelerate heart failure disease, such as coronary arterial disease. The primary pathway causing cardiac dysfunction is AGE induced diastolic dysfunction. Diastolic dysfunction is principally caused by the cross-linking of extracellular matrix proteins that are formed during the formation of AGEs. The cross-linking of extracellular matrix proteins can cause damages to normality and flexibility of the matrix proteins, leading to rigidity that induces diastolic dysfunction in heart (Hartog et al., 2007). Moreover, the activation of AGE receptors, for instance, Receptor for AGE (RAGE), is another leading cause to diastolic dysfunction. The RAGE causes the induction of fibrosis mediated by the upregulation of transforming growth factor-β (TGF-β) which further induces diastolic dysfunction (Goldin et al., 2006). In addition, activation of AGE receptors though binding to the AGE lead to a signaling cascade of stimulating NAD(P)H oxidase and increasing the ROS generation.

The second pathway leading to cardiac dysfunction is AGE induced systolic dysfunction. AGE induced systolic dysfunction is activated *via* cross-

linking of AGE peptides to low-density lipoproteins (LDL). The cross-linking with LDLs undermines the LDL particles which makes the LDL particles less susceptible to LDL-receptor uptake (Saraswat et al., 2009). Additionally, the AGE modified LDL is likely to be more receptive to macrophage uptake by AGE receptors, resulting in foam cells transformation. Foam cells transformation results in not only plaque accumulating on the endothelial wall but also inducing the expression of IL-1β and tumor necrosis factor-αmRNA (Rojas et al., 2004)

In addition, AGEs can also contribute vascular dysfunction that can cause adverse cardiac events. AGEs are able to impair vascular dysfunction by having an effecting the endothelial function and vascular compliance. AGEs can reduce the expression of the vasodilator nitric oxide (NO) and mediate the increase in production of the endothelin-1, a vasoconstrictor, causing disrupt in both endothelial and vascular function (Ahmad et al., 2006).

### 3.6.2 Neuronal cells

Deposition amyloid plaques have been characterized as one of the main cause of many neurodegenerative diseases such as Alzheimer's disease. Amyloid plaques occur as a result of protein aggregation and amyloid polymerization in which form amyloid fibrils with a cross β-structures (Christen, 2000; Papers et al., 2003).

Protein aggregation can be induced by the formation of AGEs *via* two pathways; an amyloid protein can react with a reducing sugar to form glycation and during the formation of glycation, reactive carbonyl species are

formed which are precursors of protein are cross-linking (Yan et al., 1996; Wu et al., 2011). Both pathways can lead to the cross-linking of proteins which result in a loss of the protein's original conformation and are converted into protein aggregates with a predominant β-sheet form (Gella et al., 2009). Formation of such structures causes intracellular protease-resistant, ubiquitin-proteasome-resistant deposits, and additional oxidative stress (Vitek et al., 1994).

The formation of AGEs formed by the amyloid protein as like any other protein that forms AGEs, can interact with the RAGE. This interaction causes the activation of the microglial mechanism and can induce the express of cytokines, resulting adverse cytotoxic proinflammatory effects. Moreover, RAGE can also be expressed on the surface of neurons, inducing neuronal NF-ΚB transcription factor-mediated secretion of macrophage-colony stimulating factor (M-CSF) (Cameron et al., 2005). The expressed M-CSF then reacts with its receptor C-FMS on the surface of microglia which causes cell proliferation, increase scavenger receptor, chemotaxis, apolipoprotiein E and elevated levels of oxidative stress (Bouma et al., 2003). The binding of amyloid-β fibrils and RAGE can also induce the expression of platelet endothelial cell adhesion molecule (PECAM-1), prompting the migration of monocytes across the brain's endothelial cells, an important contribution to the inflammatory response to neuronal degenerative diseases (Yan et al., 1996).

### 3.6.3 Kidney

AGEs are well known to cause modifications to protein, disturbing its main function and ultimately impairing vital organs AGEs can cause

disturbance to the kidney. Apart from the liver, in normal physiological functions, the kidney organ also contributes largely to the catabolism and metabolism of AGES, *via* the kidney's proximal tubule cells (PTCs) absorbance and glomerular filtration (Bohlender et al., 2005). However, in the state of hyperglycemia and an elevation of AGEs, addition of AGEs to PTCs can impair the function of the cells and result in tubolopathy (Saito et al., 2005). Receptors of AGEs can also cause dysfunction as its activation results in expression of substrates that will undermine normal renal function (Dan et al., 2011).

Damaged renal function occurs by thickening of the basement membrane, expansion of the mesangium, reduced filtration, and albuminuria, of which, the presence of AGEs chiefly contribute to these characteristics (Miyata et al., 1999). AGEs react through the activation of RAGE; activated RAGE in turn provokes endothelial cells to produce interleukin-6, expression of adhesion molecules, platelet-derived growth factor (PDGF), insulin growth factor-I (IGF-1) and tumor necrosis factor (TNF). These components not only cause increased generation of ROS, but also the stimulation synthesis of collagen matrix components, resulting in the thickening of basement membrane and attenuation to glomerular filtration. Additionally, expansion of the mesangial layer occurs, causing compression of capillaries and reduced surface area in which filtration occurs (Monnier et al., 2005). Supplementary, tubular epithelial-myofibroblast transdifferentiation, a disruption to morphology

of tubular epithelial cells, another leading cause to renal failure, is caused also by the activation of RAGE (Hudson et al., 2002)

### 3.6.4 Cutaneous and tissue levels

Throughout the formation of AGEs, proteins have become a major target of attack. Proteins conformation, morphology, structure and function are all modified due to protein cross-linking *via* the formation of AGES. In the cutaneous level, proteins that are mostly affected are the long lived protein, for instance, collagen and elastin. Collagen and elastin are essential for the skin's structure and function, however, due to AGEs, protein cross-linking can reduce collagen levels and debilitate the skin (Pageon et al., 2007; Song et al., 2002). Proteins at the tissue level, also due to protein cross-linking, can interact with specific and non specific AGE cell surface receptors, leading to impaired wound healing and altered intracellular events that can provoke oxidative stress and inflammation (Peppa et al., 2005).

## 3.7 AGE Inhibitors

Due to the numerous deleterious complications attributed to AGEs, the pharmacological treatment of these and alleviation of complications related to them has been a focus of investigation using a variety of therapeutics and dietary agents.

### 3.7.1 Antiglycation compounds

Several curative interventions against the glycation reaction have been developed, and have been shown to be effective at different stages of AGE formation and in alleviating AGE induced complications.

### 3.7.2 Aminoguanidine

Aminoguanidine, one of the first synthetic therapeutics used for inhibition of AGEs, works by blocking the formation of cross-links, and trapping reactive dicarbonyl intermediates from further reaction (Cameron et al., 2005). Based on its low molecular weight and highly nucleophilic character, aminoguanidine has the ability to readily react with ketones and aldehydes in order to inactivate, and prevent them from reacting with proteins (Peyroux et al., 2006). Furthermore, aminoguanidine has also been found to act as an antioxidant, quenching hydroxyl radicals and preventing lipid peroxidation (Misko et al., 1993).

### 3.7.3 Pyridoxamine

One of the natural forms of vitamin B6, pyridoxamine acts as an AGE inhibitor by strongly inhibiting the formation of carbonyl compounds derived from reducing sugars, inhibiting AGE adducts such as CML, one of the non cross-linking AGE structures (Peyroux et al., 2006; Culbertson et al., 2003). Unlike aminoguanidine, pyridoxamine works by interfering with the dicarbonyl intermediates and binding to the catalytic redox active transition metal ions (Cameron et al., 2005).

## 4. Antioxidants

Intracellular AGE formation through the oxidation of Schiff bases increases the production of free radicals and reactive carbonyl groups, forming reactive oxygen species (Dickinson et al., 2002; Wu et al., 2011). This increase in oxidative stress predisposes, and underlines the development of diabetic specific complications (Lapolla et al., 2005). Thus, agents having the ability to inhibit oxidation reactions during the formation of AGEs are of

great interest as targets for diabetes treatment. Antioxidants exist in variety of forms however natural sources of antioxidants are attracting interest due to a perceived lower toxicity, and fewer side effects than synthetic drugs.

### 4.1 EDTA

Ethylenediaminetetraacetic acid, or EDTA, is well known for its powerful ability to act as a chelating ligand for transition metal ions. Accordingly, EDTA is a suitable chelator for transition metal ions such as iron and copper, which are present in biological systems and are available to catalyze glycoxidation (Fu et al., 1994).

### 4.2 Vitamin C (ascorbic acid)

Multiple studies have shown that vitamin C, or ascorbic acid is able to prevent cellular damage from oxidative stress, through being a strong reductant and radical scavenger (Benavente-García et al., 1997). Consequently, vitamin C reduces complications induced through free radical damage, including autoxidation of glucose and glycation of structural proteins (Vinson et al., 1996). Vitamin C can also disrupt free radicals from damaging beta-cells and can induce insulin secretion in animal models (West, 2000; Upritchard et al., 2000). Rich sources of vitamin C include citrus fruits and vegetables

### 4.3 Vitamin E ($\alpha$-tocopherol)

Similar to vitamin C, vitamin E also exhibits strong antioxidant activities. Vitamin E, or $\alpha$-tocopherol, has been found to reduce the susceptibility of isolated LDL to oxidation, and reduce secretion levels of pro-inflammatory cytokines (Upritchard et al.,

2000). Thereby, vitamin E limits lipid peroxidation, thus restricting the formation of AGEs and more importantly reducing the risk of atherosclerosis (Lonn et al., 2002).

## 5. Phytochemicals

Phytochemicals are biologically active chemical compounds that can be found naturally in plants. There are mostly found to be edible and ingestion of these compounds is believed to benefit human health through modulating metabolism in a favorable manner for the prevention of chronic and degenerative diseases (Pietta, 2000). Phytochemicals can be found in many plants, and an important class of these is the polyphenols and its diverse class of substructures, which are known to exhibit many health benefits (Yao et al., 2004)

### 5.1 Polyphenols

Found in most plant material, polyphenols are a diverse class of organic molecules, which can be characterized by their multiple phenol structural rings. The amount and characteristics of the phenol structural rings influences the uniqueness and diversifies their physical, chemical and biological properties (Bravo, 1998). Based on the variety of phenol structural rings, polyphenols consist of many substructures; most common are flavonoids, phenolic acid, lignans and stilbenes.

Due to its unique phenol rings, polyphenols have shown potent properties of antioxidants. Alike to antioxidants, polyphenols can reduce oxidative stress damage through its ability to directly bind to a transitional metal such as iron that largely participates in reactive oxygen species generation, which stabilizes it to cause it to be unable to be subject to rapid oxidation (Manach et al., 2004). Moreover, polyphenols have also shown to be able to readily donate hydrogen to free radicals creating a polyphenol free radical complex which leads to stabilization to the free radical which

disallows the free radical to undergo further oxidation and initiate other free radicals (Manach et al., 2004; Perron et al., 2009)

## 5.2 Flavonoids

Like antioxidants, flavonoids have gained considerable attention due to their beneficial health properties. Flavonoids are water soluble polyphenolic compounds, that are widely found in nature and can be categorized, according to chemical structure, into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones (Pietta et al., 2000; Nijveldt et al., 2001) The general flavonoid ring structure is shown in figure 3, with rings A and B being aromatic and separated by a six-membered pyran ring C. In all cases, ring B is attached to the 2-position of ring C, and the flavonoid structure contains one, or more phenolic hydroxyl groups (Pietta et al., 2000). The number, nature and position of the subgroups (R groups) allow the differentiation of the subgroups, and examples from the same group from each other, and dictate the properties of each molecule. For example, kaempferol and quercetin are a part of the flavanol, or the 3-hydroxyflavone subgroup, and hesperitin and naringenin, which are present in citrus fruit, belong to the flavanone subgroup (Havsteen, 1983).

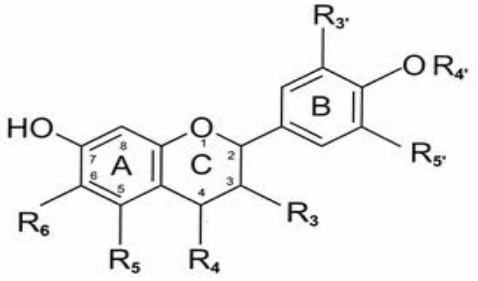



Fig. 3 The chemical structure of a flavonoid (Patel, 2008)

### 5.2.1 Biological activity

In addition to their direct antioxidant activity, flavonoids also have metal chelating abilities; assist to preserve normal cell cycle regulation, decrease inflammation, increase endothelia nitric oxide synthase activity and decrease platelet aggregation. Furthermore, flavonoids also show potential in disease prevention, especially cardiovascular disease, neurodegenerative disease and cancer (Hollman et al., 2000).

### 5.2.2 Antiglycation properties

Apart from disease prevention, multiple studies have shown that the antiglycation properties of flavonoids correlate with their antioxidant activity. It has been reported that flavonoids exhibit antiglycation properties include catechin, epicatechin, epicatechin gallate, epigallocatechin, epigallocatechin gallate, kaempferol, luteolin, quercetin, naringenin and rutin (Wu et al., 2005; Ahmed et al., 2006).

#### 5.2.2.1 Structure

Despite the antioxidant properties contributed to the flavonoids antiglycation properties, the structures of flavonoids have also shown to influence their antiglycation ability. Matsuda et al (2005) suggest that a flavonoid will effectively inhibit the formation of AGE, they must possess the structure of;

1. The more the number of hydroxyl groups at the 3'-, 4'-, 5'-, and 7'-positions increases inhibitory activities became stronger;

2. Inhibition abilities of flavones are stronger than those of flavonols, flavanones, and isoflavones;

3. Methylation or glycosylation of the 4'-hydroxyl group of flavones, flavonols, and flavanones reduces inhibition activity;

4. Methylation or glycosylation of the 3-hydroxyl group of flavonols can increase inhibition activity;

5. Glycosylation of the 7-hydroxyl group of flavones and isoflavones reduced inhibition activity.

5.2.3 Distribution of flavonoids

Ubiquitous in nature, flavonoids can be found in most plant material. Common dietary sources of flavonoids arise from fruits, vegetables, tea and beans. Fruits and vegetables like berries and tomatoes contain high amounts of quercetin. Tea, especially green tea contains high levels of catechins. Nuts and beans are usually composed of flavonoids derived from anthocyanin, and these are especially prevalent in black, and kidney beans. (Johnson et al., 2007)

6. Citrus Fruits

Amongst the various sources of phytochemicals and flavonoids, citrus fruits contain an enormous variety of flavonoids and are a major dietary source. Citrus fruits are the

primary source of vitamin C, folate, dietary fiber, flavonoids, limonoids and other bioactive compounds such as beta carotenoids (Benavente-García et al., 1997; Kawaii et al., 1999).

## 6.1 Citrus Flavonoids

Numerous flavonoids have been identified in citrus fruits, however the most common flavonoids are naringin, neoeriocitrin, hesperidin, diosmin, rutin, naringenin, eriodeictyol, hesperetin, apigenin, luteolin, disosmetin, kaempferol, quercetin and tangeretin, which all belong to the flavanone, flavone and flavanol subgroups (Rouseff et al., 1987).

## 6.2 The biological activities of citrus flavonoids

Citrus flavonoids exhibit many health benefits as shown by flavonoids from other sources, and the diversity of flavonoids that can be obtained from citrus fruits makes these an attractive source of these compounds.

### 6.2.1 Antioxidant

As in other flavonoids, citrus flavonoids show antioxidant activity; attributed to their structure, citrus flavonoids holds a hydrogen donating ability which can delocalize generated free radicals. Subsequent to this citrus flavonoids also show ability to inhibit radical formation, anti-lipoperoxidation and to act as metal chelators (Tripoli et al., 2007).

### 6.2.2 Anti-atherosclerotic

Citrus flavonoids have shown promise in the prevention of atherosclerosis. A variety of citrus flavonoids have shown to have effect on capillary fragility, a process that can increase vascular permeability, seepage of blood and plasma constituents into tissues and therefore causing inflammation. The citrus flavonoid, hesperidin, for instance, showed to act directly on the microvascular endothelium to reduce hyperpermeability and edema (Sai et al., 2007). Furthermore, citrus flavonoids also show potent abilities in the inhibition platelet aggregation by acting as an anti-adhesive and anti-aggregation against red cell clumping (Benavente-García et al., 1997). Additionally, citrus flavonoids show the ability to inhibit the generation of hyperperoxides and scavenge free radicals, they also act to inhibit oxidative modifications of LDL by macrophages and exert anti-thrombotic activity (Abeysinghe et al., 2007). A hypolipidemic effect is also exhibited by citrus flavonoids, with the dietary hesperidin inducing a hypolipidemic and hypocholesterolemic effect, decreasing the overall risk of atherosclerosis (Kim et al., 2003).

### 6.2.3 Anti-inflammation

Protective effects of inflammation by citrus flavonoids have also been well documented. Citrus flavonoids are able to inhibit productions of kinase and phosphodiesterase in cellular signal transduction and activation, compounds involved in the immune response. For example, hesperidin was able to reduce the secretion of exudates, the volume of leucocytes and histamine release. (Emim et al., 1994).

### 6.2.4 Cancer

Citrus flavonoids have been also considered as chemopreventive or act as anticancer agents. DNA damage can be caused by ultraviolet light; however this is inversely proportional by the presence of citrus flavonoids, such as naringin and rutin as they have the ability to absorb UV light (Ogata et al., 2006). Citrus flavonoids also act by increasing the detoxification process which can decrease tumor promotion at the beginning of carcinogenesis (Benavente-García et al., 1997).

### 6.2.5 Diabetes

Imputed to the variety of benefits, citrus flavonoids have also shown to be hold properties of preventing diabetes. Citrus flavonoid such as naringenin has shown to be able to reduce hepatic glucose production and inhibit of intestinal glucose uptake and renal glucose reabsorption, suggesting of naringenin can chiefly attenuates hyperglycemia (Purushotham et al., 2008; Li et al., 2006). Moreover, naringin and hesperidin were able to increase the glucokinase mRNA level which enhances glycolysis and glycogen and reduced the mRNA expression of phosphoenolpyruvate carboxykinase and glucose-6-phophatase in the liver where by decreasing the over expression of glucose (Abeysinghe et al., 2007). Furthermore, ‘Dangyuja’ one Korea’s citrus fruits enriched with flavonoids, were able to control blood glucose levels in hyperglycemia patients by inhibition of R amylase and R glucosidase in the intestinal tract consequently decrease digestion of carbohydrates (Amful et al., 2010).

### 6.2.6 Antiglycation properties

Citrus flavonoids have also shown to have antiglycation properties, although studies in this area are limited compared with other flavonoids. The citrus flavonoid naringenin has been shown to be able to have high trapping efficacy of methylglyoxal which can suppress and/or inhibit the overall formation of AGEs, as methylglyoxal is formed as an AGE precursor during the early stages of glycation (Shao, 2010). Moreover, hesperidin was able to decrease the formation of fructosamine and increase levels and activities of plasma protein thiols, resulting in inhibition of further AGE formation and decreasing the effects of protein glycation (Ibrahim, 2008).

## 7. Pomelo

Pomelo or *Citrus grandis* (L.) Osbeck, is one of the largest citrus fruit. It originates in South East Asia and can be widely found in Thailand. Pomelos are one of Thailand's cash crops, are a large part of the agricultural economy, and are widely cultivated, making this citrus fruit highly accessible to the Thai population. Pomelos are cultivated in various parts of Thailand, especially in the provinces of Samut Sakhon, Ratcha Buri, Nakhon Chaisi, Nakhon Pathom, Nakhon Nayok, Prachinburi and Nakhon Si Thammarat. Common cultivars of pomelo under cultivation in Thailand include Tar Koi, Kao Nampueng, Kao Tanggwa, Thong Dee, Tubtim Siam and Kao Yai.

Fig. 4 Pomelo – Tar Koi

Fig. 5 Pomelo – Kao Nam Pueng

Fig. 6 Pomelo – Kao Tanggwa

Fig. 7 Pomelo – Thong Dee

Fig. 8 Pomelo – Tubtim Siam

Fig. 9 Pomelo – Kao Yai

### 7.1 Flavonoid constituents of pomelo

As a part of the citrus family, pomelo fruit has shown to contain flavonoids commonly found in other citrus fruits. Flavonoids found present in pomelo are hesperidin, naringin, narirutin and neohesperidin (Ortuno et al., 1995). In addition to these a further study has shown the Thong Dee cultivar of pomelo to contain the other flavonoids, kaempferol, rutin, apigenin and quercetin (Chaiwong et al., 2009).

### 7.2 Potential antiglycation properties

From previous studies, citrus fruit extracts have been demonstrated to exert antiglycation properties, presumably through their active flavonoid content (Ramful et al., 2010). This has not yet been demonstrated for extracts of pomelo, although the presence of these components in pomelo, and the antiglycation properties of citrus flavonoids may make pomelo a potentially useful natural therapy for diabetes.

## 8. Previous studies of flavonoids compounds derived from plants on the inhibition glycation

Wu et al., (2009) determined the inhibitory effects guava leaf extracts on protein glycation by the incubation of BSA with glucose and the guava leaf extracts, with Aminoguanidine used as the positive control. Protein glycation inhibition was determined by analyses of fructosamine levels, analyses of $\alpha$ dicarbonyl and AGE analysis by fluorescence. The results show that the guava leaf extracts contain high levels of flavonoid contents, namely, quercetin and catechin. The guava leaf extracted had strong inhibition of fluorescence formed AGES and the formation of fructosamine which its inhibition was mainly attributed to its high levels of flavonoid content. (Wu et al., 2009).

Kiho et al., (2004) determined the inhibitory effects tomato on formation of AGEs by the incubation of BSA with glucose or with fructose and the tomato paste, which aminoguanidine was used as the positive control. Formation of AGEs was assessed by measurement of AGE fluorescence and the formation of fructosamine. The results show that the tomato paste extracts was able to inhibit protein glycation in both the glucose and fructose model through the presence of rutin. The inhibitory activity of the tomato extract was overall higher than that of aminoguanidine. Inhibition was based on the flavonoid content present in the tomato paste and the rutin flavonoid was the most dominant factor in such high inhibition (Kiho et al., 2004).

Wang et al., (2001) examined the inhibitory effects phytochemicals from berries and grapes on formation of AGEs by the incubation of BSA with fructose or methylglyoxal and berries extract. Formations of AGEs were monitored through AGE fluorescence and the ability to scavenge methylglyoxal and carbonyl species. The results showed that the berries were able to inhibit the overall formation of AGEs in both the fructose (79.5%) and methyglyoxal (47%) model. Most inhibition was based on the berries extract to scavenge reactive carbonyls (50.4%). The authors indication that inhibitory activity is due to the presence of cathechins and procyanidins in the berries extract (Wang et al., 2011).

Tsuji-Naito et al (2009), analyzed the inhibitory effects of Chrysanthemum species on formation of AGEs by the incubation of BSA with glucose or fructose. The results showed that the chrysanthemum extracts markedly inhibit the formation of fluorescence AGEs, the reduction of pentosidine and CML. The authors indicate that the inhibitory activity is due to the presence of apigenin, caffeic acid, lutoelin and kaempferol in the chrysanthemum extract (Tsuji-Naito et al., 2009).

# Chapter III

# Materials and Methods

## Materials

1. Plant Material
    1.1. Pomelo (Kao Yai, Kao Nampueng, Kao Tanggwa, Thong-Dee, Tub-Tim Siam, Tar Koi)
    1.2. Fruit Peeler
    1.3. Commercial Blender
2. Chemicals
    2.1. Bovine serum albumin (BSA)
    2.2. Fructose
    2.3. Phosphate Buffer Saline
    2.4. Carbonate Buffer Solution
    2.5. Nitroblue tetrazolium (NBT)
    2.6. 1– deoxy–1 morpholinofructose (DMF)
    2.7. (2,4-dinitrophenylhydrazine (DNPH)
    2.8. Ethyl acetate
    2.9. Trichloroacetic acid (TCA)
    2.10. Ethanol
    2.11. Thioflavin T reagent
    2.12. 5, 5'-dithiobisnitro benzoic acid (DTNB)
    2.13. L-cysteine
    2.14. Guanidine Hydrochloride
    2.15. $N^{\varepsilon}$-(carboxymethyl)lysine (CML) Test Kit
    2.16. 2.5M HCl

2.17. 0.01 N HCl

2.18. Acidified Methanol (0.01% (v/v) HCl in Methanol)

2.19. 1 M NaOH

2.20. 1 M HCl

2.21. Methanol

2.22. Methanol (HPLC Grade)

2.23. Acetone Nitrile (HPLC Grade0

2.24. Acetic Acid (Glacial Grade)

2.25. Hesperidin

2.26. Hesperitin

2.27. Neohesperidin

2.28. Naringin

2.29. Naringenin

2.30. Neohesperidin Dihydrochalcone

3. Equipment

3.1. Absorbance and Fluorescence microplate reader

3.2. Rotary Evaporator

3.3. Incubator

3.4. Desiccator

3.5. Lyophillizer

3.6. pH Meter

3.7. Vortex

3.8. Centrifuge

3.9. HPLC

3.10. HPLC C18 Column

3.11. Auto-pipettes

## Method

4. Source and preparation of Pomelo extract

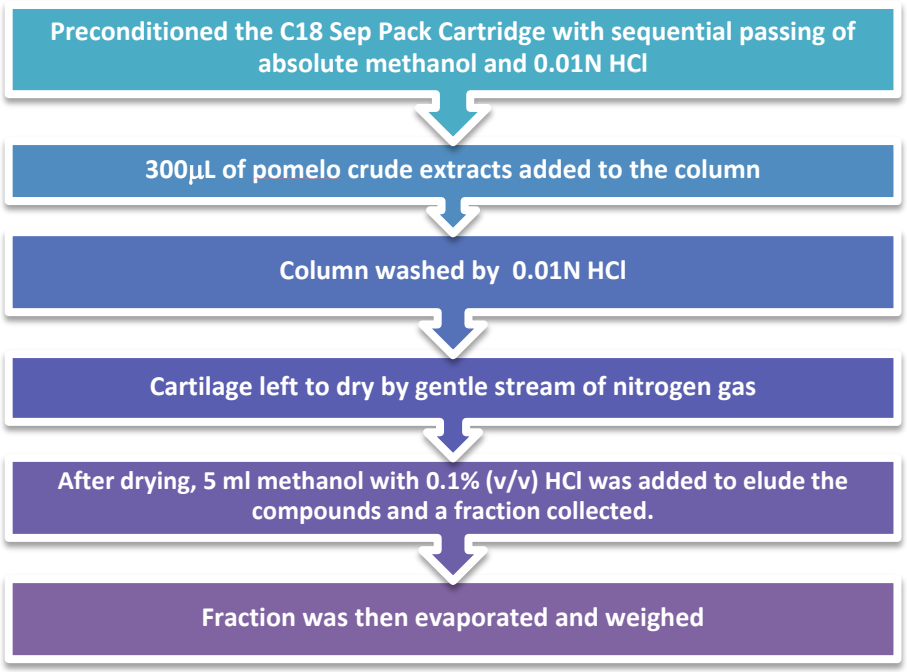
Six cultivars of pomelo were obtained from local markets in different areas of Thailand: Nakhon Pratom, Chainat, Chumporn, Surat Thani, and Pichit provinces. The pulp material of the pomelo was removed using a manual peeler and was homogenized using a commercial blender. To achieve a freeze dried extract, the pomelo pulp was then undergo lyophilization for approximately 96 hours. The freeze dried weight was determined and the material ground into a fine powder. The fine powdered pomelo extract was stored at -20°C for further analysis.

4.1. Preparation of extract

The powdered freeze-dried pomelo pulp was exhaustedly extracted using the following two step process. Methanol was added to the pomelo pulp at ratio of 1 to 10 pulp: extraction solvent and the suspension stored at 4°C for 6 consecutive days. After 4 consecutive days, the liquid phase was removed by filtration through a cheese cloth and methanol was added to the pomelo pulp. After 6 days of extraction, the liquid phases were filtered though a cheese cloth and then through a Whatman filters paper, porosity 150mm. After filtration, the liquid phases was evaporated in a rotary evaporator at 60°C for approximately 5 hours, and the batch of dried pomelo extract was stored in the dark, under vacuum desiccation, at room temperature (20-24°C).

Sugars and organic acids were removed from the powered pomleo extract that might interfere with the AGE inhibition. The powered pomelo extract was dissolved in distilled water at the ratio of 360 mg to 1.2 mL solution. The extract was purified using

C18 Sep Pack Cartridge following the procedure outlined in the schematic below(Li et al., 2006).




4.1. Quantification of flavonoid constituents

High performance liquid chromatography detection was used to quantify the flavonoid constituent in the pomelo extract. The method of analysis was adopted from that published previously (Zhang et.al., 2010) with slight modifications. Flavonoids constituent identification was determined by HPLC (Shimadzu, Kyoto, Japan) consisting of a binary pump (model LC-10A), auto-injector (model SIL-10A) and UV detector (model SPD-10A). Flavonoid separation was carried out using reversed-phase Vertic Sep™ UPS C-18 column (4.6 x 250mm, 5μm, Vertical Chromatography). The mobile phase consisted of (A): water/acetic acid (99:1, v/v) and (B): acetone nitrile/acetic acid (99:1, v/v). The gradient was as follows: 0min, 5% B; 5min, 8% B; 7min, 12% B; 12min, 18% B; 17min, 22% B; 22min, 25% B; 27min, 35% B; 37min, 53% B; 38min, 53% B; 40min, 55% B; 42min, 60% B; 57min, 80% B; 60min, 85% B; 65min, 85% B; 83min, 8% B; 85min, 5% B. The flow rate was 1.2 ml/min. The chromatograms were captured at 280nm. The flavonoids were

quantified by using naringin, naringenin, hesperitin, hesperidin, neohesperidin and neohesperidin dihydrochalcone as standard to compare against.

5. Preparation of AGEs

The formation of advanced glycation end-products involved the incubation of BSA with 0.5 M fructose to produce glycated BSA. A solution of 10mg/mL BSA and 1.1 M fructose was prepared in 0.1 M phosphate-buffered saline (PBS, pH 7.4), and the purified pomelo extract dissolved in DMSO at concentrations of 0.25 – 2 mg/mL. For comparison, aminoguanidine at concentrations of 0.5, and 1 mg/mL was used as a positive control. For the negative control, both the purified pomelo extract and aminoguanidine was absence and DMSO was added instead. As a comparison against BSA, PBS was used instead of BSA to obtain a blank control. For the blank BSA control, DMSO was added as the negative control, aminoguanidine for the positive control and the inhibition study of purified pomelo. The glycated BSA, and blank BSA was incubated at 37°C for 0, 7, 14, 21 and 28 days. After each incubation period, the glycated BSA and blank BSA solutions was assessed for advanced glycation end-products formation, and inhibition against each of the purified pomelo extracts (Tupe et al., 2010).

5.1. Fluorescence

Protein glycation was determined at an excitation wavelength of 355 nm and an emission wavelength of 460 nm using a spectrofluorometer. Glycated BSA, and blank BSA (100 μL of each) was added to each well of a 96 well plate (Tupe et al., 2010). The inhibitory effect of the purified pomelo extract on glycation-induced fluorescence formation was determined using the following equation;

$$\% \text{ Inhibition} = \frac{[(\text{Negative control} - \text{Negative blank control}) - \text{Positive control or Sample}]}{(\text{Negative control} - \text{Negative blank control})} \times 100$$

## 5.2. Fructosamine

Fructosamine is an Amadori product that is formed between fructose and an amine. The measurement of fructosamine was carried out by the use of nitroblue tetrazolium (NBT), and 1-deoxy-1-morpholino–D-fructose (DMF). DMF is a ketoamine that was used as a standard, allowing the amount of fructosamine present to be measured by direct comparison (Johnson et al., 1983)

.

Glycated BSA (10 μL) was incubated at 37°C with 2.5 mM NBT in a 96 well plate for 15 minutes. Sodium carbonate buffer add added instead of NBT as a control for comparison. The absorbance was read at 590 nm at two time intervals, the first reading was at 10 minutes and the second was at 15 minutes. From the absorbance readings, the concentration of fructosamine present was obtained by comparison with the DMF standard curve (concentration range 1.25 – 5 mg/mL).

## 5.3. Protein Oxidation

### 5.3.1. Carbonyl content

For the assessment of protein oxidation, protein carbonyl content was measured. To quantify the protein carbonyl content, 2, 4-dinitrophenylhydrazine (DNPH) was used to react with protein carbonyl groups to form a 2, 4-dinitrophenylhydrazone (Ardestani et al., 2007; Schmitt et al., 2005)

Glycated BSA (100 μL) with 10mM DNPH (400 μL) in a plastic tube was incubated at room temperature for 60 minutes and at every 15 minutes each tube was vortexed to allow efficient mixing. 2.5M HCl (400 μL) was added

instead of DNPH as the control for comparison. Following incubation, 20% TCA (500 μL) was added to each tube and the mixture vortexed to thoroughly mix the contents. The tubes were then incubated in ice for 5 minutes. Then, the tubes were centrifuged at 10,000 rpm for 10 minutes at 4°C. After centrifugation, the supernatant was discarded and the pellets was re-suspended in 500 μL of 10% TCA, and then incubated on ice for 5 minutes. Following incubation, the tubes were centrifuged at 10,000 rpm for 10 minutes at 4°C. From centrifugation, the supernatant was discarded and the pellets were re-suspended in 500 μL of ethanol/ethyl acetate (1/1) mixture and mixed thoroughly by vortexing. After mixing, the tubes were centrifuged at 10,000 rpm for 10 minutes at 4°C. After discarding of the supernatant, the process of re-suspending the pellets and centrifugation was repeated two more times. Then, 250 μL of 6M guanidine hydrochloride was added to each tube and vortexed thoroughly to mix. To remove any leftover debris, the tubes were centrifuged at 10,000 rpm for 10 minutes at 4°C to settle the contents. Finally, 110 μL of the supernatant solution was transferred to each well of a 96 well plate. Absorbance was measured at 370 nm for protein carbonyl amounts.

Due to the possibility of proteins being lost during manual washing, protein levels were determined from the final pellet after the washing procedure. From the controlled glycated BSA (glycated BSA and 2.5M HCl) supernatant solution, 10 μL of the solution was transferred into each well of a 96 well plate, and 90 μL of 6M guanidine hydrochloride was added to each. After mixing, the absorbance was read at 280 nm to determine protein concentration.

To determine the carbonyl protein concentration a BSA standard curve over a concentration range of 0.125 – 2mg/mL was used. A solution of BSA dissolved in 6M guanidine hydrochloride at a concentration of 2 mg/mL was used as a

stock solution, and serial dilutions to achieve concentrations of 0.125 – 1 mg/mL was made. The absorbance was read at 220 nm for each of these solutions to generate a standard curve. From absorbance readings total carbonyl content was determined by the following equations;

Protein carbonyl (nmol/ml) = $[(abs_{sample}-abs_{blank}) / (*0.011\ \mu M^{-1})](250\mu l/100\mu l)$

Protein concentration (mg/ml) = $[(abs_{280}$- (y-intercept)/slope)] x **2.5 x ***10

Carbonyl content (nmol/mg) = (Carbonyl nmol/ml)/ (protein mg/ml)

*Extinction coefficient of DNPH at 370 nm = 22,000 $M^{-1}cm^{-1}(0.022\mu M^{-1}cm^{-1})$

**Correction factor = (250µl/100µl) = 2.5

***Dilution factor = (100µl/10µl) = 10

5.3.2. Thiol Groups

To further evaluate glycoxidation, the amount of reduced protein thiol was determined. Glycoxidation can cause modifications of the thiol groups, such as formation of disulfide bonds and intermolecular aggregations in albumin. As a result, the quantity of protein thiol groups in the sample would decrease as the formation of advanced glycation end products increases over time.

For assaying the content of protein thiol present, DNTB (5,5'-dithiobis-(2-nitrobenzoic acid)), or Ellman's Reagent, was used to detect the thiol groups present in solution. From the assay, the thiol concentration was calculated in units of nmol thiol/mg protein, from comparison with a L-cysteine calibration curve showing concentrations between 50 – 3.12 nM (Tupe et al., 2010; Ardestani et al., 2007).

Glycated BSA (70 μL) was incubated at room temperature for 15 minutes with 2.5 mM DNTB (130 μL). PBS was added instead of 2.5 mM DNTB for comparison as a control. After incubation, the absorbance of the solution was read at 410 nm. From the absorbance readings, protein thiol estimation was calculated from the L-cysteine calibration curve at concentrations between 3.12 – 50 nM.

5.4. Determination of β-amyloid structures

To quantify the formation of amyloid fibrils resulting from protein cross linking during glycation, Thioflavin T dye (4-(3,6-dimethyl-1,3-benzothiadiazol-3-ium-2-yl)-N,N dimethylaniline chloride, ThT) was used in a fluorescence assay. Thioflavin T dye is a benzothiazole dye that exhibits enhanced fluorescence upon binding to amyloid fibrils.

Glycated BSA (10 μL) was incubated at room temperature with the ThT dye (100 μL) for 60 minutes. PBS was added to other tubes of glycated BSA instead of the ThT dye as a control for comparison. The ThT fluorescence emission was measured with excitation at 435 nm, and emission at 485 nm, which gives the direct indication of the β-amyloid structures levels (Tupe et al., 2010).

5.5. $N^{\varepsilon}$-(carboxymethyl)lysine (CML)

To identify formation of an AGE structure, $N^{\varepsilon}$-(carboxymethyl)lysine (CML) was quantified using a CML-ELISA kit. CML structures are formed between an α-dicarbonyl compound and amino acid residues.

Quantification is based upon a quantitative competitive enzyme immunoassay. Glycated BSA samples were diluted to a 1 to 4 ratio with PBS, mixed with 200 μL of anti-CML-adduct monoclonal antibody and 100 μL of solution placed into each well of a 96 well plate. Mixtures in the 96 well plate were incubated at 37°C for 2 hours. After incubation, the wells were washed twice with PBS solution (250 μL) to remove any unbound substances, and an HRP conjugated polyclonal antibody specific for mouse IgG polyclonal antibody (200 μL) was added. After the addition of the HRP conjugated antibody, the plates were washed again three times with PBS solution to remove any unbound antibody HRP conjugate. A substrate reagent ($H_2O_2$-tetramethylbenzidine) (100 μL) was then be added to react with the bound conjugate. After a set reaction time (20 minutes), the reaction was stopped by the addition of 1N $H_2SO_4$ solution, and the absorbance of the yellow product taken at 450nm. The absorbance value was inversely proportional to the concentration of CML adduct. For comparison, a standard curve was constructed by plotting the absorbance values of the samples against CML-adduct concentrations and concentrations of CML present in the samples were determined.

6. Statistical Analysis

Data were expressed as mean ± SEM for all experimental measurements, based on triplicate results (n=3). Significant differences were determined using the one way analysis of variance (ANOVA) statistical assessment followed by post-hoc testing with Tukey's Range Test. $P < 0.05$ was considered to be statistically significant.

# Chapter IV

# Results

## 1. Quantification of flavonoids constituents in pomelo cultivars

Six flavonoids were used as the standard compound including naringin, hesperidin, neohesperidin, neohesperidin dihydrochalcone, naringenin and hesperitin. Figure 17 shows the standard curve of six flavonoids with concentrations of 1.95 – 250 μg/ml. Flavonoids concentrations in the pomelo extract were quantified directly by HPLC. Figure 10 - 15 shows the chromatogram of six pomelo cultivar extract.

Among the extracts, naringin and naringenin were present in all pomelo cultivars. Especially naringin was the most widely distributed with a varying amount in the six pomelo cultivars. The highest contents of naringin was in Tar Koi with 36.79 ± 0.25 μg/mg extract and the lowest content was in Kao Nam Pueng with 2.34 ± 0.11 μg/mg extract, whereas, hesperitin was only detected in Thong Dee with 3.13 ± 0.01μg/mg extract, respectively.

**Figure 10** – The HPLC chromatogram of Tar Koi

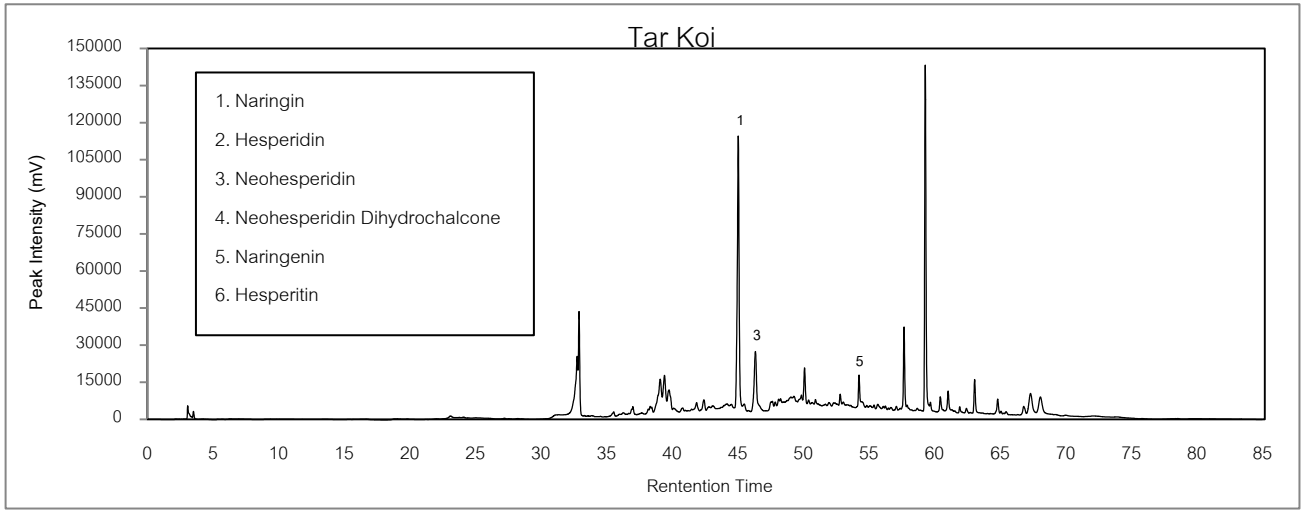



**Figure 11** – The HPLC chromatogram of Kao Nam Pueng

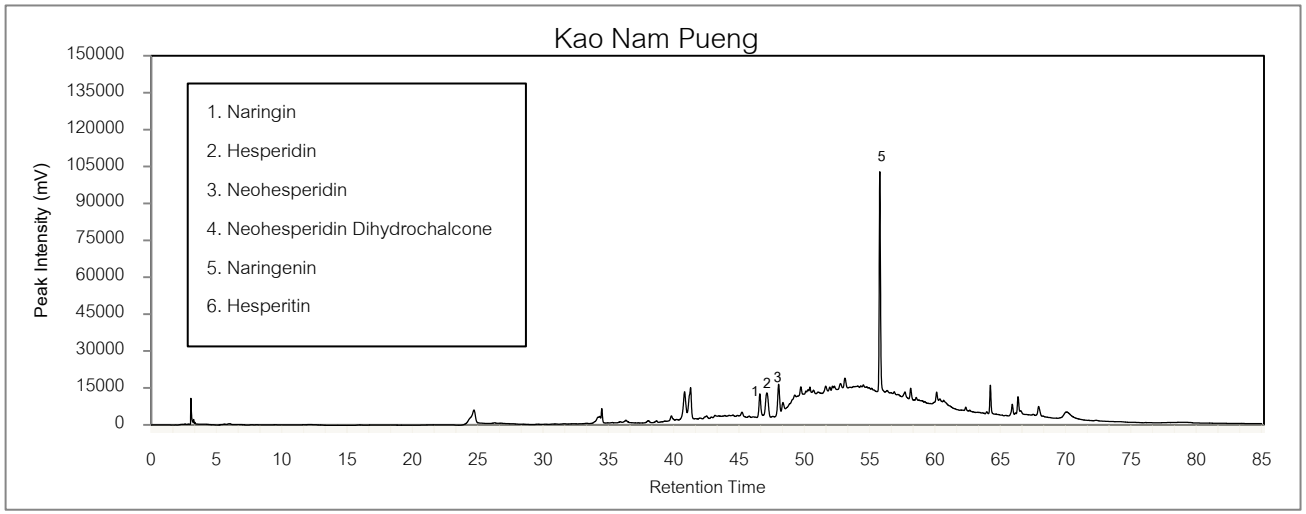


**Figure 12** – The HPLC chromatogram of Kao Tanggwa

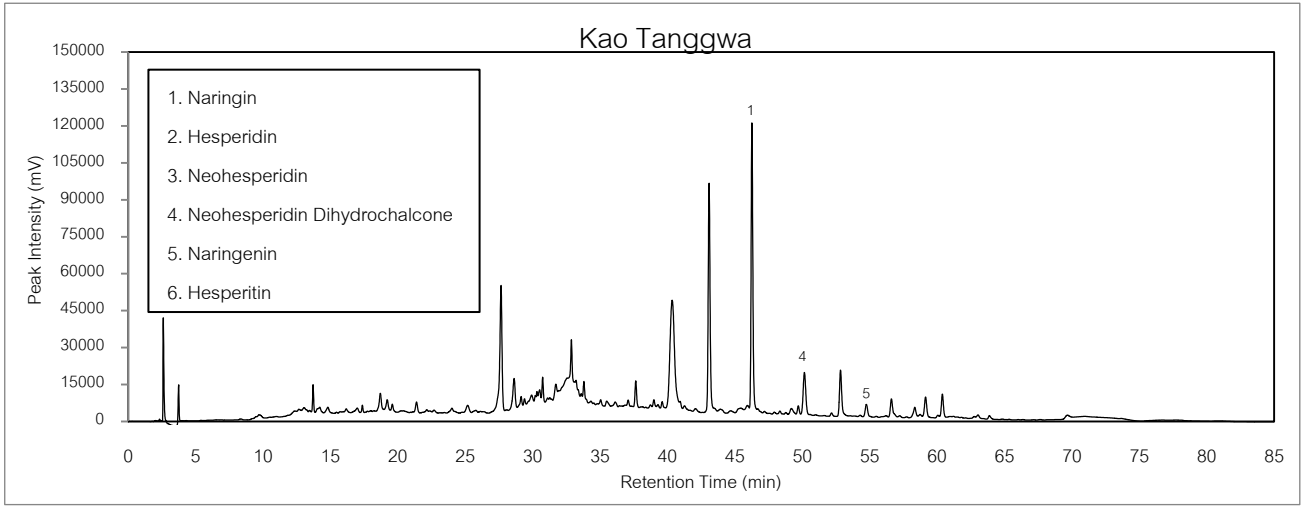



**Figure 13** – The HPLC chromatogram of Thong Dee

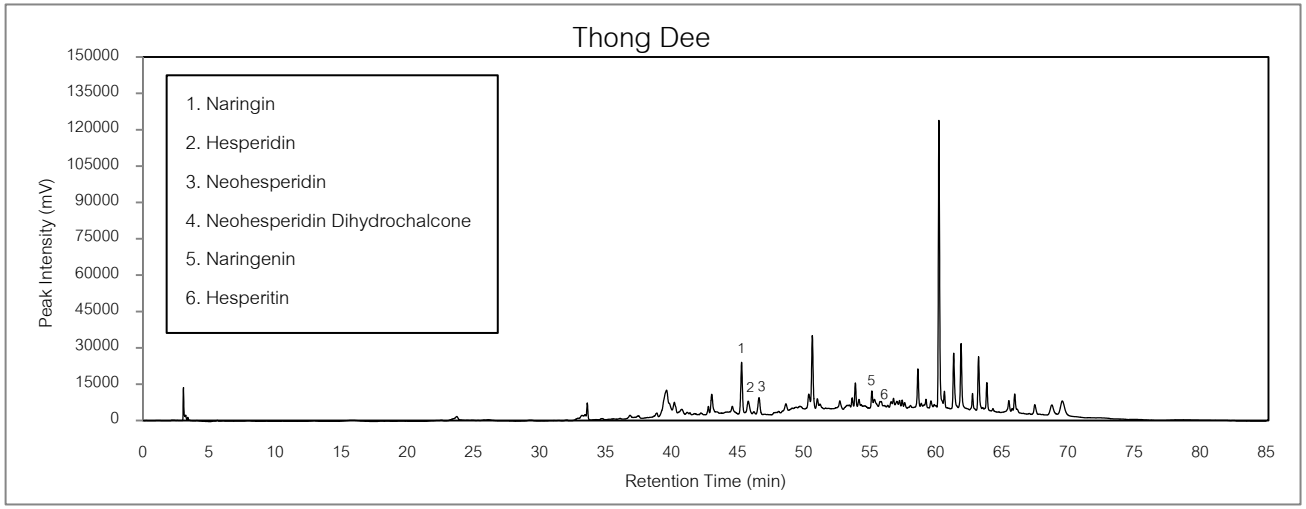


**Figure 14** – The HPLC chromatogram of Tubtim Siam

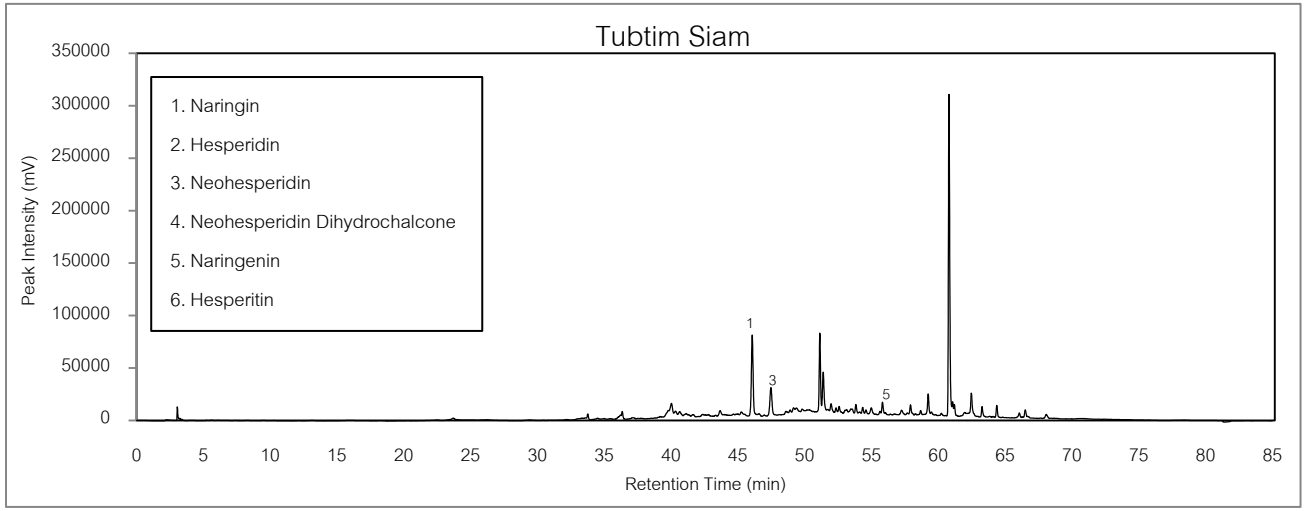



**Figure 15** – The HPLC chromatogram of Kao Yai

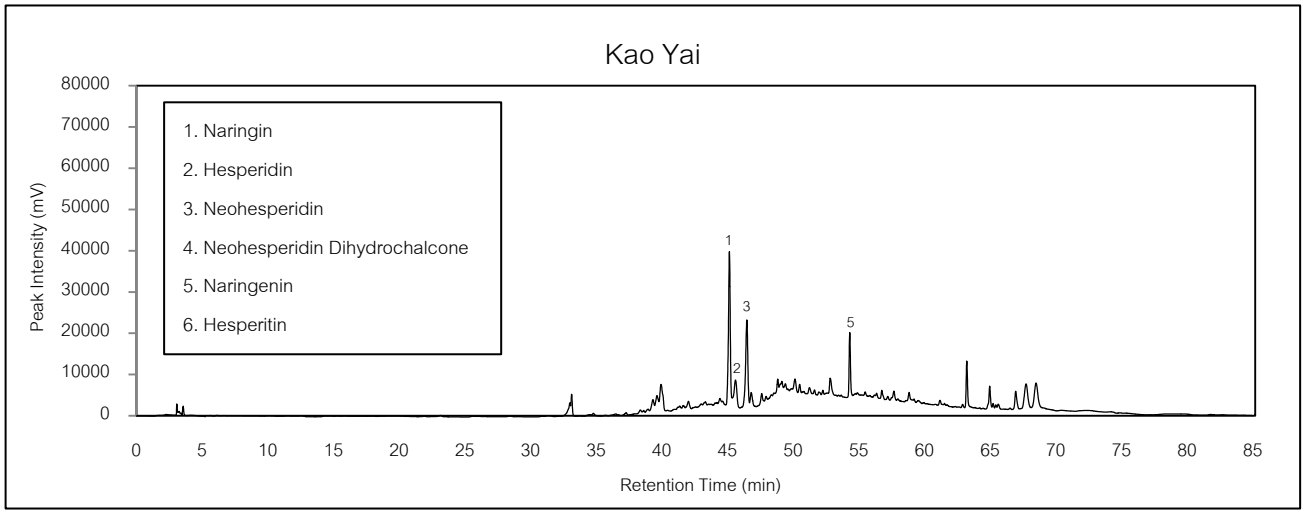


**Figure 16** – The HPLC chromatogram of flavonoid standards (250 μg/ml)

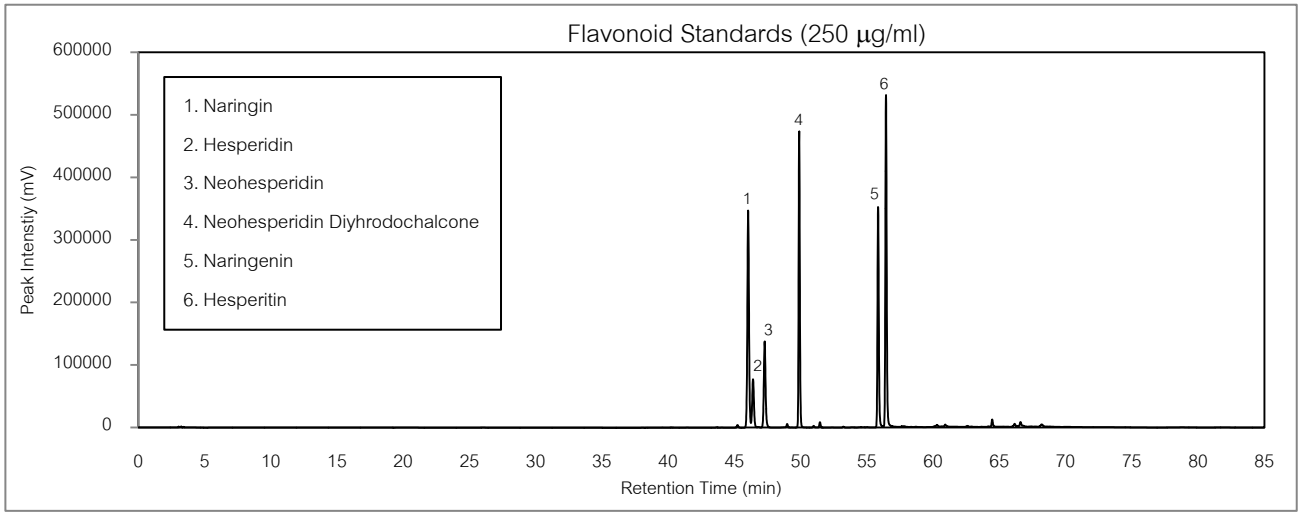
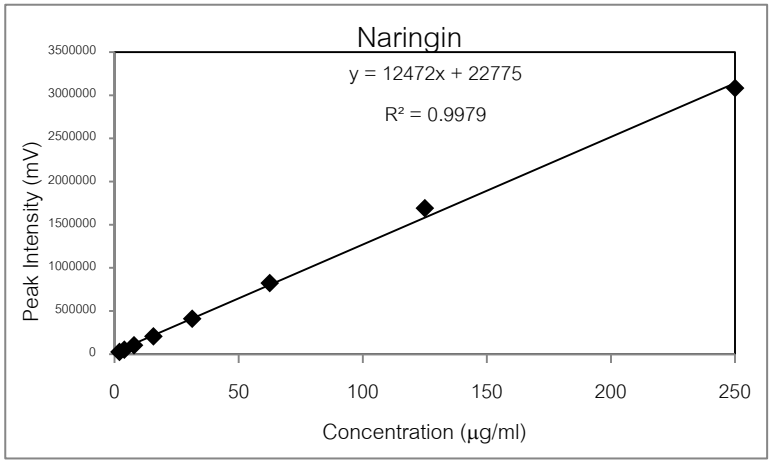
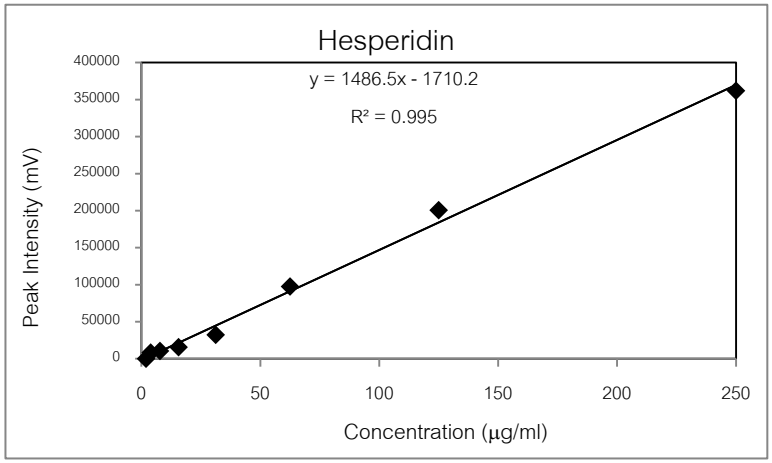
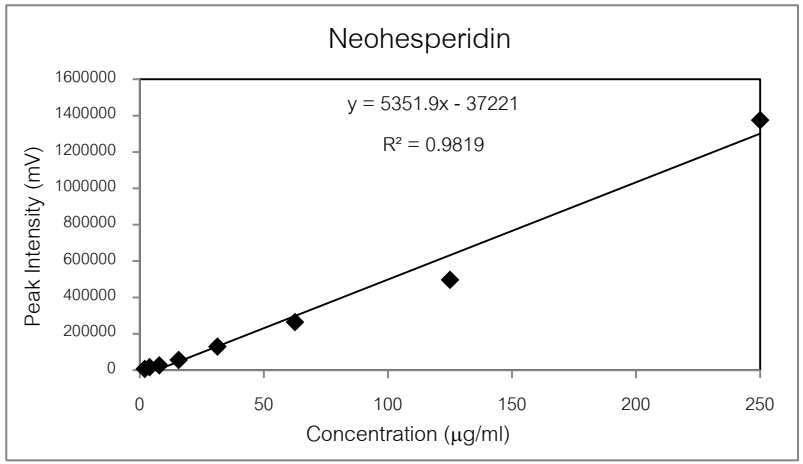
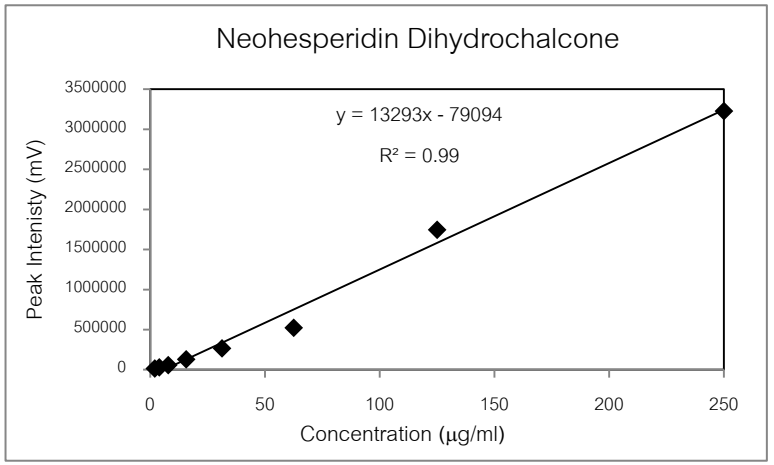


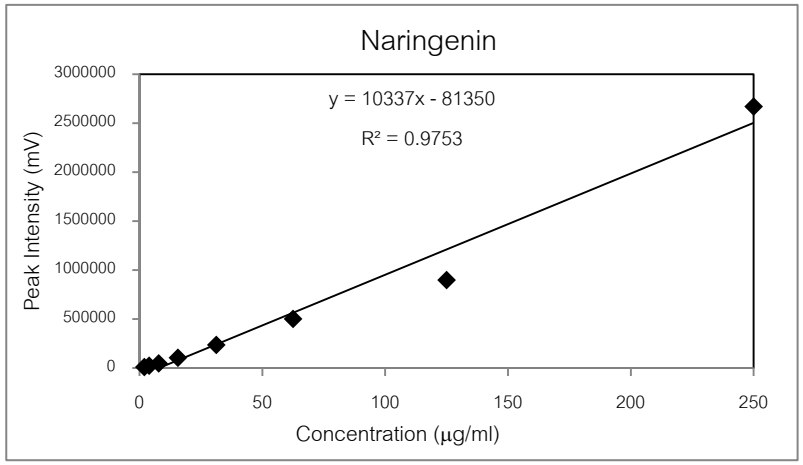

**Figure 17** – The standard curves of flavonoids




Neohesperidin
y = 5351.9x - 37221
R² = 0.9819
Peak Intensity (mV)
Concentration (µg/ml)
0
200000
400000
600000
800000
1000000
1200000
1400000
1600000
0
50
100
150
200
250


Neohesperidin Dihydrochalcone
y = 13293x - 79094
R² = 0.99
Peak Intenisty (mV)
Concentration (µg/ml)
0
500000
1000000
1500000
2000000
2500000
3000000
3500000
0
50
100
150
200
250


Naringenin
y = 10337x - 81350
R² = 0.9753
Peak Intensity (mV)
Concentration (µg/ml)
0
500000
1000000
1500000
2000000
2500000
3000000
0
50
100
150
200
250

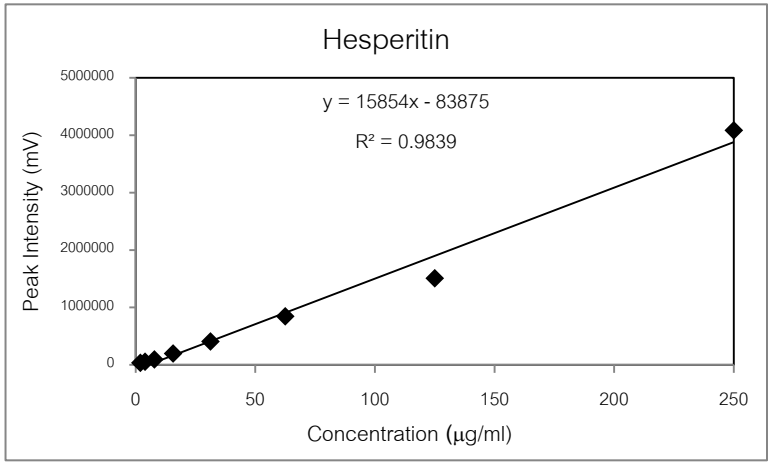

Hesperitin
y = 15854x - 83875
R² = 0.9839
Peak Intensity (mV)
Concentration (µg/ml)
0
1000000
2000000
3000000
4000000
5000000
0
50
100
150
200
250

Table 1 – Flavonoid peak area under the curve of six pomelo cultivars (AUC)

| Peak area under the curve (AUC) | | | | | | |
|---|---|---|---|---|---|---|
| Flavonoids | Pomelo Cultivar | | | | | |
| | Tar Koi | Kao Nam Pueng | Kao Tanggwa | Thong Dee | Tubtim Siam | Kao Yai |
| Naringin | 1,046,089 ± 231 | 81,204.33 ± 294 | 1,036,626.22 ± 210 | 225,667.33 ± 462 | 679,122.84 ± 321 | 319,996.33 ± 253 |
| Hesperidin | ND | 137,231.67 ± 481 | ND | ND | ND | 69,460.667.22 ± 272 |
| Neohesperidin | 353,813.67 ± 372 | 119,077.12 ± 124 | ND | 76,836.55 ± 150 | 280,612.67 ± 195 | 233,103.31 ± 213 |
| Neohesperidin Dihydrochalcone | ND | ND | 245,780 ± 371 | 218,335.70 ± 158 | ND | ND |
| Naringenin | 71,515.67 ± 431 | 303,623.33 ± 128 | 171,607.70 ± 118 | 143,810.33 ± 215 | 98,420.33 ± 238 | 108,853.67 ± 126 |
| Hesperitin | ND | ND | ND | 156,86.333 ± 135 | ND | ND |

Data are expressed as mean ± SEM, n =3. ND = not detectable.

**Table 2** – Flavonoid concentrations of six pomelo cultivars (μg/ml)

| Flavonoid concentration (μg/ml) | | | | | | |
|---|---|---|---|---|---|---|
| Flavonoids | Pomelo Cultivar | | | | | |
| | Tar Koi | Kao Nam Pueng | Kao Tanggwa | Thong Dee | Tubtim Siam | Kao Yai |
| Naringin | 82.58 ± 0.68 | 4.68 ± 0.22 | 81.29 ± 0.39 | 16.27 ± 0.25 | 52.63 ± 0.88 | 23.83 ± 0.43 |
| Hesperidin | ND | 45.56 ± 0.65 | ND | 20.17 ± 0.23 | ND | 24.08 ± 0.23 |
| Neohesperidin | 73.58 ± 0.49 | 29.52 ± 0.30 | ND | 21.53 ± 0.07 | 59.84 ± 0.36 | 50.93 ± 0.23 |
| Neohesperidin Dihydrochalcone | ND | ND | 24.54 ± 0.66 | ND | ND | ND |
| Naringenin | 14.78 ± 0.30 | 59.05 ± 0.80 | 24.47 ± 0.98 | 21.78 ± 0.29 | 14.8 ± 0.09 | 18.40 ± 0.38 |
| Hesperitin | ND | ND | ND | 6.27 ± 0.03 | ND | ND |
| Total (6) | 170.94 ± 0.21 | 138.81 ± 0.11 | 130.30 ± 0.30 | 86.02 ± 0.18 | 127.27 ± 0.22 | 117.24 ± 0.14 |

Data are expressed as mean ± SEM, n =3. ND = not detectable.

Table 3 – Flavonoid concentrations of six pomelo cultivars (μg/mg extract)

| Flavonoid concentration (μg/mg extract) | | | | | | |
|---|---|---|---|---|---|---|
| Flavonoids | Pomelo Cultivar | | | | | |
| | Tar Koi | Kao Nam Pueng | Kao Tanggwa | Thong Dee | Tubtim Siam | Kao Yai |
| Naringin | 41.29 ± 0.43 | 2.34 ± 0.11 | 40.65 ± 0.39 | 8.13 ± 0.13 | 26.31 ± 0.44 | 11.90 ± 0.21 |
| Hesperidin | ND | 22.78 ± 0.33 | ND | 10.08 ± 0.12 | ND | 12.04 ± 0.12 |
| Neohesperidin | 36.79 ± 0.25 | 14.76 ± 0.15 | ND | 10.76 ± 0.03 | 29.92 ± 0.18 | 25.4 ± 0.12 |
| Neohesperidin Dihydrochalcone | ND | ND | 12.27 ± 0.66 | ND | ND | ND |
| Naringenin | 7.39 ± 0.15 | 29.52 ± 0.40 | 12.23 ± 0.98 | 10.89 ± 0.15 | 7.40 ± 0.04 | 9.20 ± 0.19 |
| Hesperitin | ND | ND | ND | 3.13 ± 0.01 | ND | ND |
| Total (6) | 85.47 ± 0.11 | 69.40 ± 0.05 | 65.15 ± 0.15 | 43.01 ± 0.09 | 63.60 ± 0.11 | 58.54 ± 0.07 |

Data are expressed as mean ± SEM, n =3. ND = not detectable.

## 2. The effects of pomelo extract against protein glycation

### 2.1. Determination of AGE formation

The level of AGE formation formed by fluorescence AGEs and the effect of pomelo extract were determined at day 7, 14, 21 and 28. The increased fluorescent intensity indicates a progressive accumulation of AGEs in all incubations of BSA + fructose (negative control), BSA + fructose + aminoguanidine (positive control) and also in the pomelo extracts during the experimental period (Table 4, 6, 8, 10, 12, 14). The results showed that all pomelo extracts markedly inhibited fluorescent intensity in a concentration-dependent manner. A lowered fluorescent intensity indicates the reduction of AGE formation. When compared to the BSA + fructose, it was found that all pomelo cultivars at all concentrations (0.25, 0.50, 0.75, 1.00, 1.50, 2.00 mg/ml) exhibited a significantly lowered fluorescent intensity ($P<0.01$). Compared to aminoguanidine however, the aminoguanidine (1.00 mg/ml) was able to exhibit a lower fluorescent intensity than the pomelo extracts ($P<0.01$).

Apart from a lowered fluorescent intensity, the pomelo extracts also exerted several percentages of inhibition. The percentage of inhibition exerted by pomelo cultivar extracts are shown in Table 4 – 15 and Figure 18 – 22. On day 7, the highest percentage of inhibition was exerted by the pomelo cultivar Kao Yai at concentration of 1.00 mg/ml, which it was able to exert a similar amount of inhibition as aminoguanidine (1.00 mg/ml) by 91.00±0.03 and 91.13±2.73, respectively. At day 14 and day 21, the highest percentage of inhibition was still exerted by pomelo cultivar Kao Yai at 1.00 mg/ ml with 88.85±0.95 and 87.01±0.66, which was rather alike to aminoguanidine (1.00 mg/ml) that exerted 90.10±0.42 and 87.36±0.80, respectively. At day 28, the pomelo cultivar Kao Yai remained as the cultivar to exert the highest percentage of inhibition and the inhibition of 86.31±0.48 was higher than that of aminoguanidine (1.00 mg/ml) that exerted 84.23±0.60. As for the other pomelo cultivars at 1.00 mg/ml, they did not exert a similar or a higher inhibition than aminoguanidine (1.00 mg/ml).

**Table 4 –** The effect of pomelo cultivar (Tar Koi) on the formation of fluorescence AGE at day 7, 14, 21 and 28.

| Fluorescence AGE (355, 460 nm) | | | | |
|---|---|---|---|---|
| | Day 7 | Day 14 | Day 21 | Day 28 |
| BSA | 10,392.22 ± 72 | 21,038.45 ± 102 | 42,248.44 ± 98 | 65,894.12 ± 65 |
| BSA + Fructose | 217,244.67 ± 321 | 457,295.34 ± 231 | 594,314.45 ± 346 | 628,006.76 ± 563 |
| BSA + Fructose + TK 0.25 mg/ml | 69,422.33 ± 834$^{\#*}$ | 126,206.45 ± 532$^{\#*}$ | 221,894.57 ± 348$^{\#*}$ | 262,717.34 ± 325$^{\#*}$ |
| BSA + Fructose + TK 0.50 mg/ml | 64,348.45 ± 391$^{\#*}$ | 117,808.54 ± 351$^{\#*}$ | 211,030.65 ± 231$^{\#*}$ | 252,437.00 ± 356$^{\#*}$ |
| BSA + Fructose + TK 0.75 mg/ml | 60,472.43 ± 467$^{\#*}$ | 108,049.32 ±354$^{\#*}$ | 199,650.45 ± 326$^{\#*}$ | 242,154.23 ± 375$^{\#*}$ |
| BSA + Fructose + TK 1.00 mg/ml | 58,562.33 ± 984$^{\#*}$ | 100,440.22 ± 242$^{\#*}$ | 186,163.33 ± 323$^{\#*}$ | 232,397.56 ± 361$^{\#*}$ |
| BSA + Fructose + TK 1.50 mg/ml | 50,070.56 ± 423$^{\#*}$ | 92,215.15 ± 351$^{\#*}$ | 176,654.67 ±421$^{\#*}$ | 208,995.76 ± 329$^{\#*}$ |
| BSA + Fructose + TK 2.00 mg/ml | 44,203.67 ± 478$^{\#*}$ | 81,454.45 ± 362$^{\#*}$ | 161,902.30 ± 213$^{\#*}$ | 171,119.78 ± 354$^{\#*}$ |
| BSA + Fructose + AG 0.50 mg/ml | 39,130.21 ± 843$^{\#}$ | 63,388.56 ± 153$^{\#}$ | 114,182.12 ± 523$^{\#}$ | 161,529.34 ± 843$^{\#}$ |
| BSA + Fructose + AG 1.00 mg/ml | 12,807.29 ± 473 | 42,917.12 ± 322 | 62,121.87 ± 587 | 81,464.64 ± 473 |

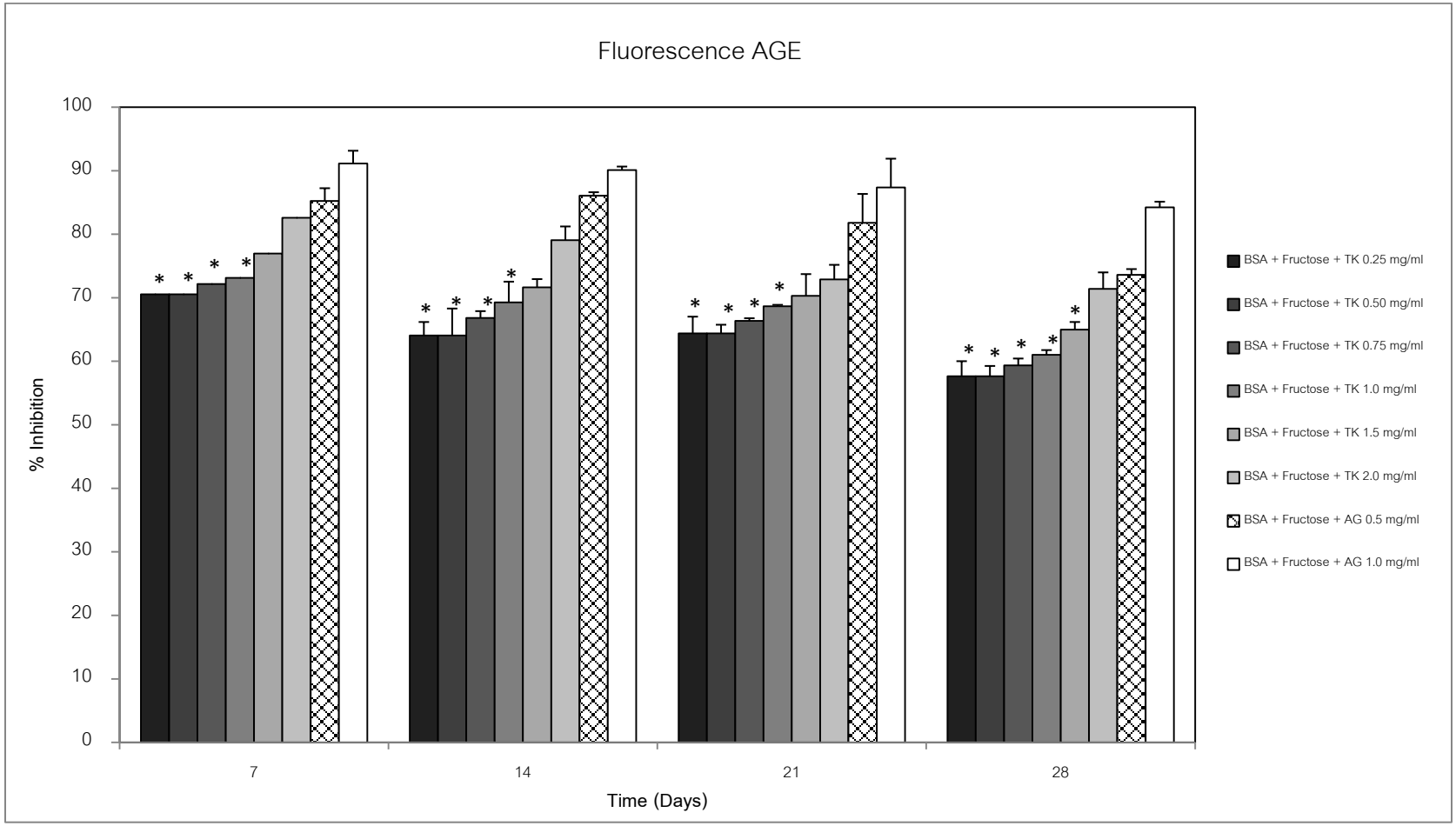
Data are expressed as mean ± SEM, n =3.$^{\#}P < 0.01$ compared to BSA+Fructose and * $P < 0.05$ compared to aminoguanidine 1.00 mg/ml.

**Table 5 –** The percentage inhibition of pomelo cultivar (Tar Koi) on the formation of fluorescence AGE at day 7, 14, 21 and 28.

| | % Inhibition | | | |
|---|---|---|---|---|
| | Day 7 | Day 14 | Day 21 | Day 28 |
| BSA + Fructose + TK 0.25 mg/ml | 68.09 ± 1.30* | 61.34 ± 2.63* | 62.57 ± 2.37* | 56.03 ± 2.51* |
| BSA + Fructose + TK 0.50 mg/ml | 70.54 ± 0.84* | 64.05 ± 1.36* | 64.4 ± 1.62* | 57.65 ± 1.82* |
| BSA + Fructose + TK 0.75 mg/ml | 72.16 ± 1.37* | 66.83 ± 0.43* | 66.35 ± 1.09* | 59.35 ± 1.83* |
| BSA + Fructose + TK 1.00 mg/ml | 73.13 ± 0.83* | 69.28 ± 0.22* | 68.67 ± 0.76* | 61.02 ± 1.46* |
| BSA + Fructose + TK 1.50 mg/ml | 76.96 ± 0.41 | 71.63 ± 3.43* | 70.3 ± 1.22* | 64.97 ± 2.93* |
| BSA + Fructose + TK 2.00 mg/ml | 82.59 ± 1.76 | 79.06 ± 2.31 | 72.88 ± 2.60 | 71.40 ± 2.04 |
| BSA + Fructose + AG 0.50 mg/ml | 85.23 ± 1.89 | 86.06 ± 0.56 | 81.81± 4.54 | 73.62 ± 0.90 |
| BSA + Fructose + AG 1.00 mg/ml | 91.13 ± 2.73 | 90.10 ± 0.42 | 87.36 ± 0.80 | 84.23 ± 0.60 |

Data are expressed as mean ± SEM, n =3.*$P < 0.05$ compared to aminoguanidine 1.00 mg/ml.

**Figure 18 –** The percentage inhibition of pomelo cultivar (Tar Koi) on the formation of fluorescence AGE at day 7, 14, 21 and 28.




Data are expressed as mean ± SEM, n =3.*$P < 0.05$ compared to aminoguanidine 1.00 mg/ml.

**Table 6 –** The effect of pomelo cultivar (Kao Nam Pueng) on the formation of fluorescence AGE at day 7, 14, 21 and 28.

| Fluorescence AGE (355, 460 nm) | | | | |
|---|---|---|---|---|
| | Day 7 | Day 14 | Day 21 | Day 28 |
| BSA | 10,392.22 ± 72 | 21,038.45 ± 102 | 42,248.44 ± 98 | 65,894.12 ± 65 |
| BSA + Fructose | 217,244.67 ± 321 | 457,295.34 ± 231 | 594,314.45 ± 346 | 628,006.76 ± 563 |
| BSA + Fructose + KNP 0.25 mg/ml | 69,799.22 ± 581$^{\#*}$ | 137,385.34 ± 398$^{\#*}$ | 163,774.12 ± 479$^{\#*}$ | 191,550.41 ± 167$^{\#*}$ |
| BSA + Fructose + KNP 0.50 mg/ml | 56,783.41 ± 389$^{\#*}$ | 120,552.44 ± 367$^{\#*}$ | 141,931.33 ± 387$^{\#*}$ | 164,559.23 ± 182$^{\#*}$ |
| BSA + Fructose + KNP 0.75 mg/ml | 43,671.23 ± 375$^{\#*}$ | 94,247.56 ± 351$^{\#*}$ | 113,079.44 ± 301$^{\#*}$ | 123,448.45 ± 323$^{\#*}$ |
| BSA + Fructose + KNP 1.00 mg/ml | 27,876.54 ± 213$^{\#}$ | 87,681.43 ± 312$^{\#*}$ | 101,946.67 ± 438$^{\#*}$ | 115,642.32 ± 336$^{\#*}$ |
| BSA + Fructose + KNP 1.50 mg/ml | 21,737.21 ± 348$^{\#}$ | 38,231.33 ± 329$^{\#}$ | 49,047.65 ± 193$^{\#}$ | 57,781.76 ± 483$^{\#}$ |
| BSA + Fructose + KNP 2.00 mg/ml | 8,660.23 ± 385$^{\#*}$ | 26,749.67 ± 471$^{\#*}$ | 36,246.32 ± 398$^{\#}$ | 48,550.40 ± 121$^{\#}$ |
| BSA + Fructose + AG 0.50 mg/ml | 39,130.21 ± 843$^{\#}$ | 63,388.56 ± 153$^{\#}$ | 114,182.12 ± 523$^{\#}$ | 161,529.34 ± 843$^{\#}$ |
| BSA + Fructose + AG 1.00 mg/ml | 12,807.29 ± 473 | 42,917.12 ± 322 | 62,121.87 ± 587 | 81,464.64 ± 473 |

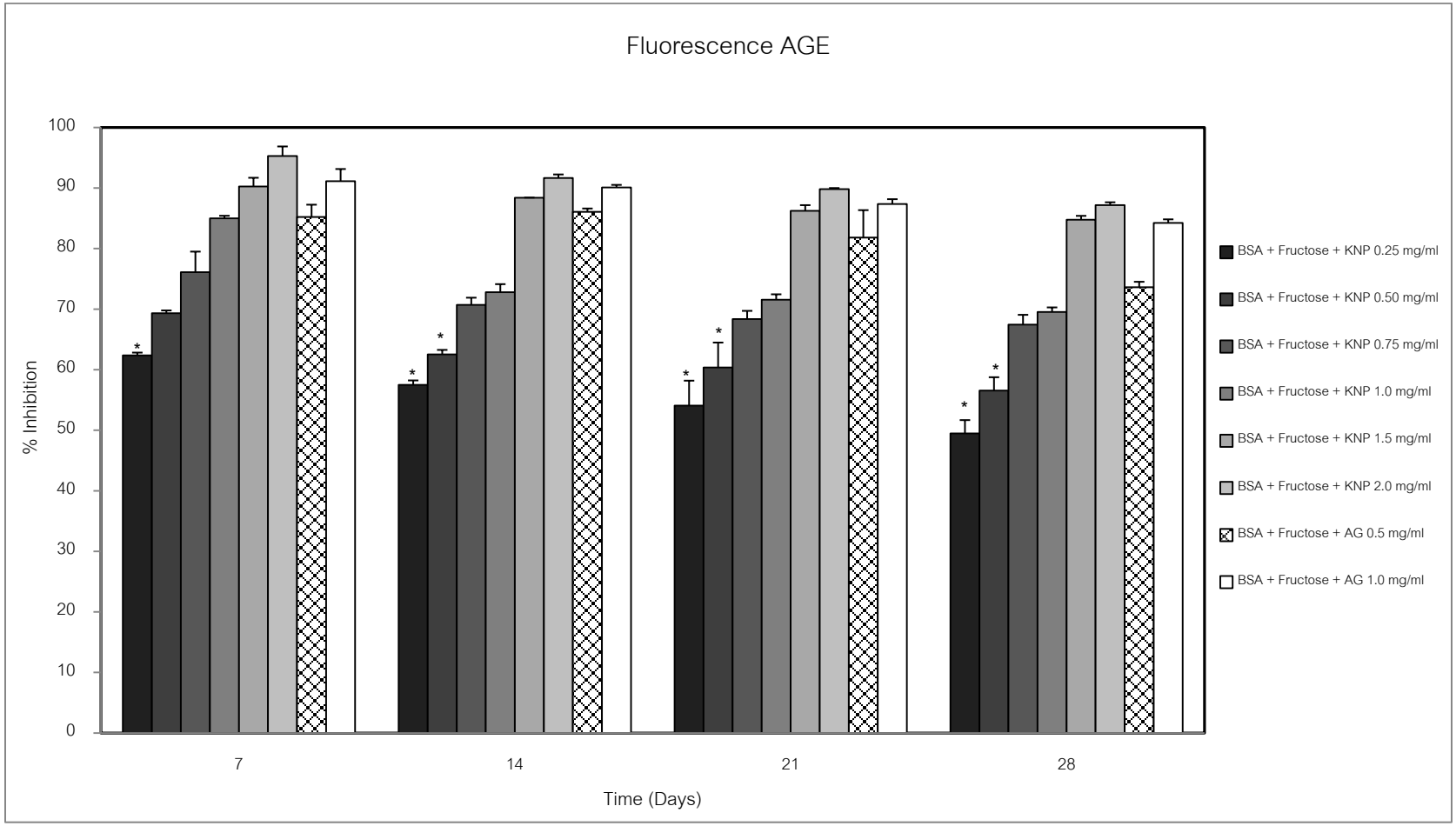
Data are expressed as mean ± SEM, n =3. $^{\#}P < 0.01$ compared to BSA+Fructose and $^{*}P < 0.05$ compared to aminoguanidine 1.00 mg/ml.

**Table 7 –** The percentage inhibition of pomelo cultivar (Kao Nam Pueng) on the formation of fluorescence AGE at day 7, 14, 21 and 28.

| | % Inhibition | | | |
|---|---|---|---|---|
| | Day 7 | Day 14 | Day 21 | Day 28 |
| BSA + Fructose + KNP 0.25 mg/ml | 62.37 ± 0.21* | 57.49 ± 0.20* | 54.07 ± 0.43* | 49.48 ± 0.31* |
| BSA + Fructose + KNP 0.50 mg/ml | 69.33 ± 0.01* | 62.53 ± 0.32* | 60.36 ± 0.79* | 56.56 ± 0.50* |
| BSA + Fructose + KNP 0.75 mg/ml | 76.11 ± 0.05 | 70.70 ± 0.97* | 68.35 ± 0.82* | 67.43 ± 0.55* |
| BSA + Fructose + KNP 1.00 mg/ml | 85 ± 0.15 | 72.80 ± 0.20* | 71.55 ± 0.33* | 69.52 ± 0.40* |
| BSA + Fructose + KNP 1.50 mg/ml | 90.26 ± 0.12 | 88.39 ± 0.10 | 86.22 ± 0.24 | 84.75 ± 0.18 |
| BSA + Fructose + KNP 2.00 mg/ml | 95.29 ± 0.13 | 91.64 ± 0.71 | 89.81 ± 0.21 | 87.17 ± 0.25 |
| BSA + Fructose + AG 0.50 mg/ml | 85.23 ± 0.22 | 86.06 ± 0.56 | 81.81± 0.14 | 73.62 ± 0.90 |
| BSA + Fructose + AG 1.00 mg/ml | 91.13 ± 0.73 | 90.10 ± 0.42 | 87.36 ± 0.01 | 84.23 ± 0.60 |

Data are expressed as mean ± SEM, n =3.*$P < 0.05$ compared to aminoguanidine 1.00 mg/ml.

**Figure 19** – The percentage inhibition of pomelo cultivar (Kao Nam Pueng) on the formation of fluorescence AGE at day 7, 14, 21 and 28.




Data are expressed as mean ± SEM, n =3.* $P < 0.05$ compared to aminoguanidine 1.00 mg/ml.

**Table 8 –** The effect of pomelo cultivar (Kao Tanggwa) on the formation of fluorescence AGE at day 7, 14, 21 and 28.

| Fluorescence AGE (355, 460 nm) | | | | |
|---|---|---|---|---|
| | Day 7 | Day 14 | Day 21 | Day 28 |
| BSA | 10,392.22 ± 72 | 21,038.45 ± 102 | 42,248.44 ± 98 | 65,894.12 ± 65 |
| BSA + Fructose | 217,244.67 ± 321 | 457,295.34 ± 231 | 594,314.45 ± 346 | 628,006.76 ± 563 |
| BSA + Fructose + TG 0.25 mg/ml | 53,683.23 ± 366$^{\#*}$ | 256,764.32 ± 279$^{\#*}$ | 267,672.43 ± 329$^{\#*}$ | 291,419.31 ± 194$^{\#*}$ |
| BSA + Fructose + TG 0.50 mg/ml | 51,884.35 ± 387$^{\#*}$ | 248,008.50 ± 472$^{\#*}$ | 253,058.12 ± 653$^{\#*}$ | 284,914.43 ± 173$^{\#*}$ |
| BSA + Fructose + TG 0.75 mg/ml | 39,685.55 ± 325$^{\#*}$ | 221,736.42 ± 481$^{\#*}$ | 222,777.45 ± 472$^{\#*}$ | 216,724.67 ± 181$^{\#*}$ |
| BSA + Fructose + TG 1.00 mg/ml | 27,873.40 ± 631$^{\#*}$ | 196,739.87 ± 370$^{\#*}$ | 178,047.65 ± 367$^{\#*}$ | 170,459.33 ± 139$^{\#*}$ |
| BSA + Fructose + TG 1.50 mg/ml | 17,402.67 ± 732$^{\#}$ | 97,325.12 ± 351$^{\#*}$ | 119,765.44 ± 353$^{\#*}$ | 147,476.34 ± 291$^{\#*}$ |
| BSA + Fructose + TG 2.00 mg/ml | 13,303.33 ± 421 $^{\#}$ | 78,656.33 ± 462$^{\#*}$ | 88,901.65 ± 531$^{\#*}$ | 107,765.55 ± 483$^{\#*}$ |
| BSA + Fructose + AG 0.50 mg/ml | 39,130.21 ± 843$^{\#}$ | 63,388.56 ± 153$^{\#}$ | 114,182.12 ± 523$^{\#}$ | 161,529.34 ± 843$^{\#}$ |
| BSA + Fructose + AG 1.00 mg/ml | 12,807.29 ± 473 | 42,917.12 ± 322 | 62,121.87 ± 587 | 81,464.64 ± 473 |

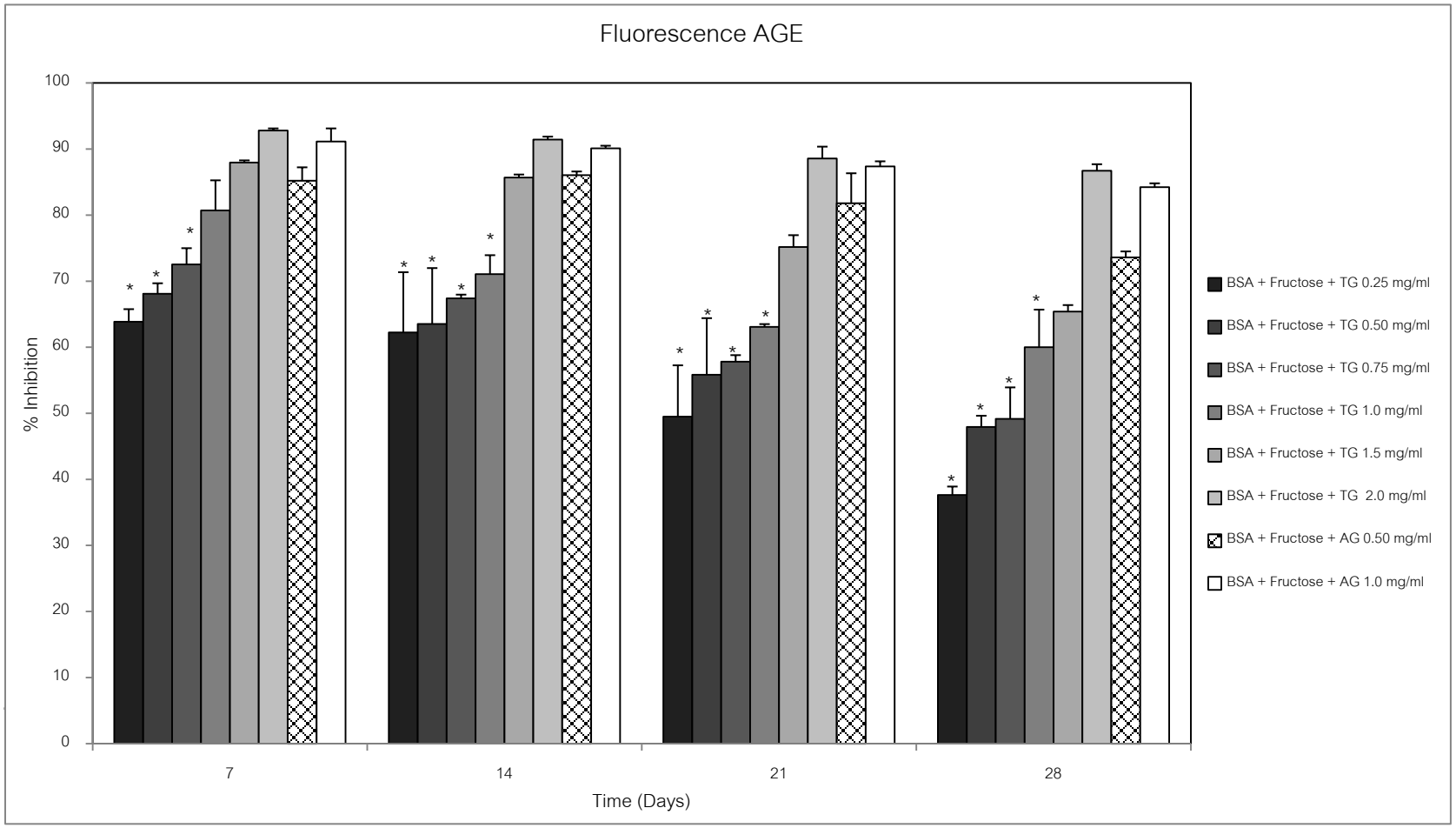
Data are expressed as mean ± SEM, n =3. $^{\#}P < 0.01$ compared to BSA+Fructose and * $P < 0.05$ compared to aminoguanidine 1.00 mg/ml.

**Table 9 –** The percentage inhibition of pomelo cultivar (Kao Tanggwa) on the formation of fluorescence AGE at day 7, 14, 21 and 28.

| | % Inhibition | | | |
|---|---|---|---|---|
| | Day 7 | Day 14 | Day 21 | Day 28 |
| BSA + Fructose + TG 0.25 mg/ml | 63.89 ± 1.92 * | 62.24 ± 9.13* | 49.50 ± 7.78* | 37.63 ± 1.30* |
| BSA + Fructose + TG 0.50 mg/ml | 68.1 ± 1.60* | 63.52 ± 8.48* | 55.83 ± 8.59* | 47.94 ± 1.71* |
| BSA + Fructose + TG 0.75 mg/ml | 72.54 ± 2.47* | 67.39 ± 0.59* | 59.81 ± 1.01* | 49.15 ± 4.79* |
| BSA + Fructose + TG 1.00 mg/ml | 80.72 ± 4.56 | 71.06 ± 2.89* | 63.08 ± 0.44* | 60.01 ± 5.70* |
| BSA + Fructose + TG 1.50 mg/ml | 87.96 ± 2.37 | 85.69 ± 2.20 | 75.17 ± 6.73 | 65.40 ± 8.99* |
| BSA + Fructose + TG 2.00 mg/ml | 92.80 ± 0.34 | 91.43 ± 0.47 | 88.57 ± 1.81 | 86.72 ± 1.00 |
| BSA + Fructose + AG 0.50 mg/ml | 85.23 ± 0.22 | 86.06 ± 0.56 | 81.81± 0.56 | 73.62 ± 0.90 |
| BSA + Fructose + AG 1.00 mg/ml | 91.13 ± 0.73 | 90.10 ± 0.42 | 87.36 ± 0.01 | 84.23 ± 0.60 |

Data are expressed as mean ± SEM, n =3.*$P < 0.05$ compared to aminoguanidine 1.00 mg/ml.

**Figure 20** – The percentage inhibition of pomelo cultivar (Kao Tanggwa) on the formation of fluorescence AGE at day 7, 14, 21 and 28




Data are expressed as mean ± SEM, n =3.*$P < 0.05$ compared to aminoguanidine 1.00 mg/ml.

**Table 10 –** The effect of pomelo cultivar (Thong Dee) on the formation of fluorescence AGE at day 7, 14, 21 and 28.

| Fluorescence AGE (355, 460 nm) | | | | |
|---|---|---|---|---|
| | Day 7 | Day 14 | Day 21 | Day 28 |
| BSA | 10,392.22 ± 72 | 21,038.45 ± 102 | 42,248.44 ± 98 | 65,894.12 ± 65 |
| BSA + Fructose | 217,244.67 ± 321 | 457,295.34 ± 231 | 594,314.45 ± 346 | 628,006.76 ± 563 |
| BSA + Fructose + TD 0.25 mg/ml | 70,041.23 ± 382$^{\#*}$ | 113,114.45 ± 372$^{\#*}$ | 226,605.32 ± 173$^{\#*}$ | 237,982.34 ± 431$^{\#*}$ |
| BSA + Fructose + TD 0.50 mg/ml | 55,847.55 ± 231$^{\#*}$ | 84,347.60 ± 134$^{\#*}$ | 180,886.49 ± 450$^{\#*}$ | 197,593.44 ± 451$^{\#*}$ |
| BSA + Fructose + TD 0.75 mg/ml | 49,604.67 ± 684$^{\#*}$ | 76,638.34 ± 103$^{\#*}$ | 163,671.32 ± 356$^{\#*}$ | 160,473.56 ± 381$^{\#*}$ |
| BSA + Fructose + TD 1.00 mg/ml | 26,521.76 ± 472$^{\#}$ | 54,484.87 ± 102$^{\#*}$ | 131,033.43 ± 311$^{\#*}$ | 134,240.23 ± 246$^{\#*}$ |
| BSA + Fructose + TD 1.50 mg/ml | 18,591.70 ± 531$^{\#*}$ | 45,650.70 ± 329$^{\#}$ | 100,418.56 ± 110$^{\#*}$ | 109,930.56 ± 254$^{\#}$ |
| BSA + Fructose + TD 2.00 mg/ml | 14,928.54 ± 631$^{\#*}$ | 31,641.12 ± 831$^{\#}$ | 76,847.54 ± 128$^{\#*}$ | 94,023.40 ± 273$^{\#}$ |
| BSA + Fructose + AG 0.50 mg/ml | 39,130.21 ± 843$^{\#}$ | 63,388.56 ± 153$^{\#}$ | 114,182.12 ± 523$^{\#}$ | 161,529.34 ± 843$^{\#}$ |
| BSA + Fructose + AG 1.00 mg/ml | 12,807.29 ± 473 | 42,917.12 ± 322 | 62,121.87 ± 587 | 81,464.64 ± 473 |

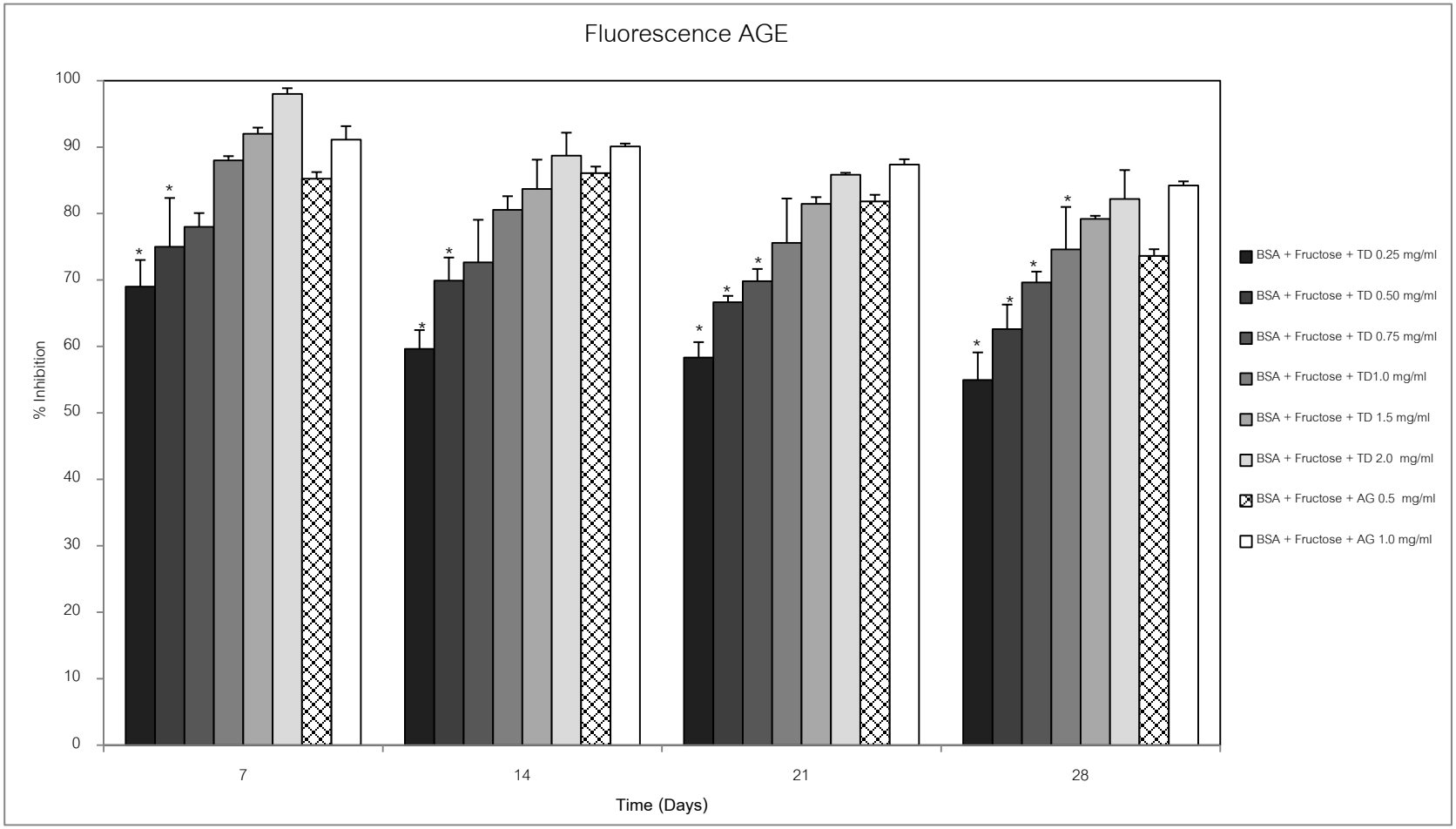
Data are expressed as mean ± SEM, n =3. $^{\#}P < 0.01$ compared to BSA+Fructose and $^{*}P < 0.05$ compared to aminoguanidine 1.00 mg/ml.

**Table 11 –** The percentage inhibition of pomelo cultivar (Thong Dee) on the formation of fluorescence AGE at day 7, 14, 21 and 28.

| | % Inhibition | | | |
|---|---|---|---|---|
| | Day 7 | Day 14 | Day 21 | Day 28 |
| BSA + Fructose + TD 0.25 mg/ml | 69.12 ± 0.82* | 59.63 ± 2.30* | 58.34 ± 0.13* | 54.96 ± 0.21* |
| BSA + Fructose + TD 0.50 mg/ml | 75.37 ± 3.47* | 69.90 ± 0.94* | 66.66 ± 0.09* | 62.6 ± 0.55* |
| BSA + Fructose + TD 0.75 mg/ml | 78.13 ± 0.40 | 72.65 ± 1.84 | 69.81 ± 1.62* | 63.63 ± 0.06* |
| BSA + Fructose + TD 1.00 mg/ml | 88.31 ± 2.04 | 80.56 ± 0.66 | 75.59 ± 0.39 | 71.59 ± 0.87* |
| BSA + Fructose + TD 1.50 mg/ml | 91.80 ± 4.39 | 83.71 ± 1.01 | 81.45 ± 0.46 | 79.19 ± 0.12 |
| BSA + Fructose + TD 2.00 mg/ml | 97.83 ± 0.45 | 88.71 ± 0.29 | 85.83 ± 0.33 | 82.2 ± 0.20 |
| BSA + Fructose + AG 0.50 mg/ml | 85.23 ± 1.89 | 86.06 ± 0.56 | 81.81± 4.54 | 73.62 ± 0.90 |
| BSA + Fructose + AG 1.00 mg/ml | 91.13 ± 2.73 | 90.10 ± 0.42 | 87.36 ± 0.80 | 84.23 ± 0.60 |

Data are expressed as mean ± SEM, n =3.*$P < 0.01$ compared to aminoguanidine 1.00 mg/ml.

**Figure 21** – The percentage inhibition of pomelo cultivar (Thong Dee) on the formation of fluorescence AGE at day 7, 14, 21 and 28.




Data are expressed as mean ± SEM, n =3.*$P < 0.01$ compared to aminoguanidine 1.00 mg/ml.

**Table 12 –** The effect of pomelo cultivar (Tubtim Siam) on the formation of fluorescence AGE at day 7, 14, 21 and 28.

| Fluorescence AGE (355, 460 nm) | | | | |
|---|---|---|---|---|
| | Day 7 | Day 14 | Day 21 | Day 28 |
| BSA | 10,392.22 ± 72 | 21,038.45 ± 102 | 42,248.44 ± 98 | 65,894.12 ± 65 |
| BSA + Fructose | 217,244.67 ± 321 | 457,295.34 ± 231 | 594,314.45 ± 346 | 628,006.76 ± 563 |
| BSA + Fructose + TS 0.25 mg/ml | 77,132.23 ± 311$^{\#*}$ | 138,659.34 ± 354$^{\#*}$ | 240,393.23 ± 490$^{\#*}$ | 279,763.40 ± 410$^{\#*}$ |
| BSA + Fructose + TS 0.50 mg/ml | 69,011.45 ± 399$^{\#*}$ | 129,966.54 ± 564$^{\#*}$ | 207,202.43 ± 426$^{\#*}$ | 266,069.52 ± 235$^{\#*}$ |
| BSA + Fructose + TS 0.75 mg/ml | 61,036.34 ± 213$^{\#*}$ | 126,348.40 ± 635$^{\#*}$ | 191,465.40 ± 495$^{\#*}$ | 249,833.33 ± 491$^{\#*}$ |
| BSA + Fructose + TS 1.00 mg/ml | 44,425.45 ± 312 | 118,900.33 ± 573$^{\#*}$ | 169,155.12 ± 342$^{\#*}$ | 211,703.45 ± 184$^{\#*}$ |
| BSA + Fructose + TS 1.50 mg/ml | 37,523.18 ± 221$^{\#}$ | 103,454.35 ± 388$^{\#*}$ | 119,284.34 ± 492$^{\#*}$ | 204,498.12 ± 341$^{\#*}$ |
| BSA + Fructose + TS 2.00 mg/ml | 31,428.34 ± 493$^{\#}$ | 75,751.54 ± 381$^{\#}$ | 101,686.43 ± 392$^{\#}$ | 147,962.12 ± 139$^{\#*}$ |
| BSA + Fructose + AG 0.50 mg/ml | 39,130.21 ± 843$^{\#}$ | 63,388.56 ± 153$^{\#}$ | 114,182.12 ± 523$^{\#}$ | 161,529.34 ± 843$^{\#}$ |
| BSA + Fructose + AG 1.00 mg/ml | 12,807.29 ± 473 | 42,917.12 ± 322 | 62,121.87 ± 587 | 81,464.64 ± 473 |

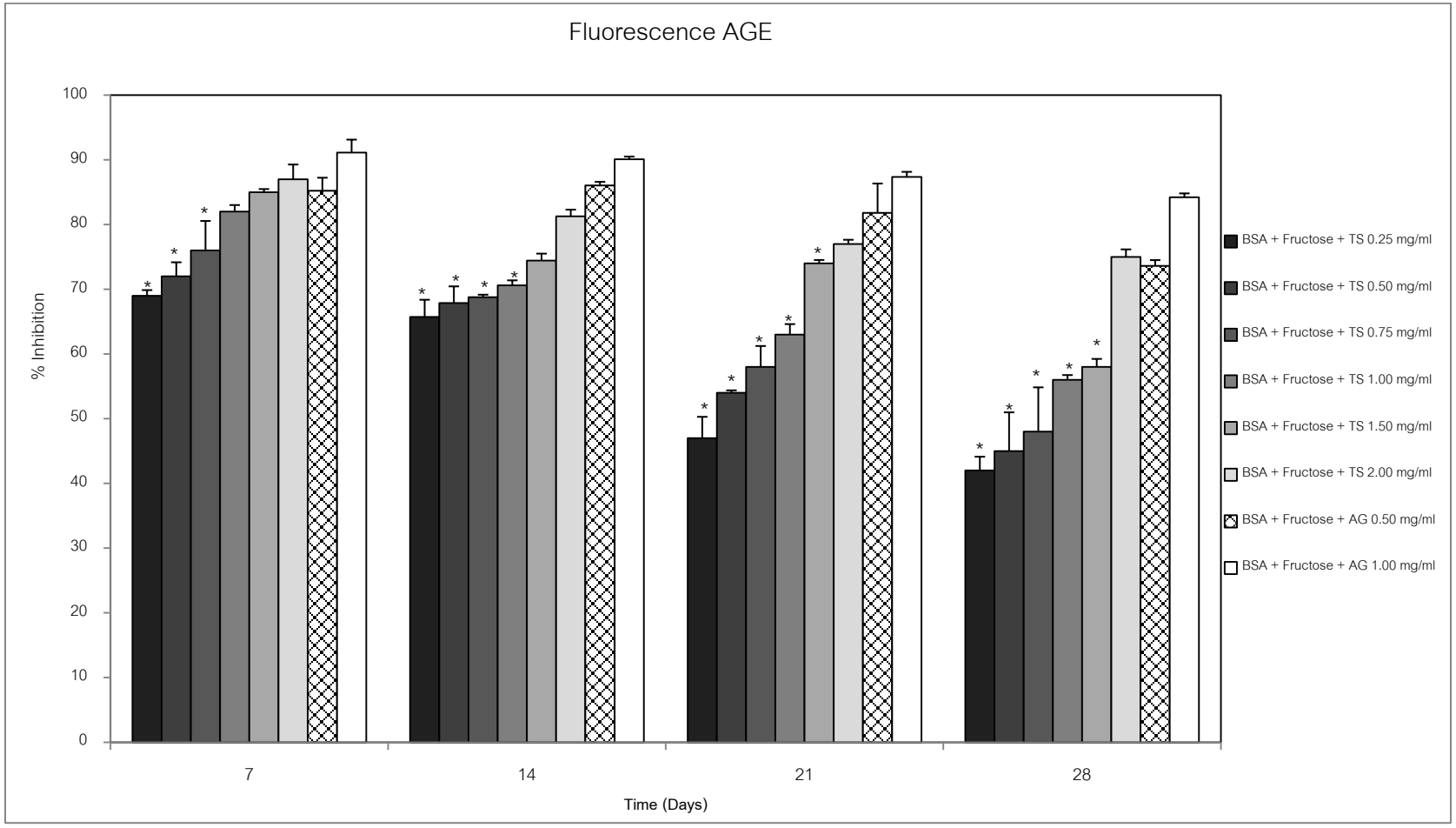
Data are expressed as mean ± SEM, n =3. $^{\#}P < 0.01$ compared to BSA+Fructose and * $P < 0.05$ compared to aminoguanidine 1.00 mg/ml.

**Table 13 –** The percentage inhibition of pomelo cultivar (Tubtim Siam) on the formation of fluorescence AGE at day 7, 14, 21 and 28.

| | % Inhibition | | | |
|---|---|---|---|---|
| | Day 7 | Day 14 | Day 21 | Day 28 |
| BSA + Fructose + TS 0.25 mg/ml | 69.19 ± 0.89* | 65.74 ± 2.65* | 46.78 ± 3.30* | 41.91 ± 2.14* |
| BSA + Fructose + TS 0.50 mg/ml | 72.43 ± 2.18* | 67.88 ± 2.58* | 54.13 ± 0.39* | 44.75 ± 6.00* |
| BSA + Fructose + TS 0.75 mg/ml | 75.62 ± 4.57 | 68.78 ± 0.40* | 57.61 ± 3.25* | 48.12 ± 6.86* |
| BSA + Fructose + TS 1.00 mg/ml | 82.25 ± 1.03 | 70.62 ± 0.77* | 62.55 ± 1.63* | 56.04 ± 0.75* |
| BSA + Fructose + TS 1.50 mg/ml | 85.01 ± 0.50 | 74.44 ± 1.08 | 73.59 ± 0.54* | 68.32 ± 1.25* |
| BSA + Fructose + TS 2.00 mg/ml | 87.45 ± 2.29 | 81.28 ± 1.04 | 77.49 ± 0.66 | 75.28 ± 1.18 |
| BSA + Fructose + AG 0.50 mg/ml | 85.23 ± 1.89 | 86.06 ± 0.56 | 81.81± 4.54 | 73.62 ± 0.90 |
| BSA + Fructose + AG 1.00 mg/ml | 91.13 ± 2.73 | 90.10 ± 0.42 | 87.36 ± 0.80 | 84.23 ± 0.60 |

Data are expressed as mean ± SEM, n =3.* $P < 0.05$ compared to aminoguanidine 1.00 mg/ml.

**Figure 22** – The percentage inhibition of pomelo cultivar (Tubtim Siam) on the formation of fluorescence AGE at day 7, 14, 21 and 28.




Data are expressed as mean ± SEM, n =3.*$P < 0.05$ compared to aminoguanidine 1.00 mg/ml.

**Table 14 –** The effect of pomelo cultivar (Kao Yai) on the formation of fluorescence AGE at day 7, 14, 21 and 28.

| Fluorescence AGE (355, 460 nm) | | | | |
|---|---|---|---|---|
| | Day 7 | Day 14 | Day 21 | Day 28 |
| BSA | 10,392.22 ± 72 | 21,038.45 ± 102 | 42,248.44 ± 98 | 65,894.12 ± 65 |
| BSA + Fructose | 217,244.67 ± 321 | 457,295.34 ± 231 | 594,314.45 ± 346 | 628,006.76 ± 563 |
| BSA + Fructose + KY 0.25 mg/ml | 64,206.33 ± 273#* | 196,701.23 ± 342#* | 243,575.29 ± 391#* | 314,203.23 ± 273#* |
| BSA + Fructose + KY 0.50 mg/ml | 37,161.21 ± 573#* | 80,589.36 ± 882#* | 150,579.23 ± 294#* | 178,800.43 ± 573#* |
| BSA + Fructose + KY 0.75 mg/ml | 33,624.45 ± 576#* | 58,178.76 ± 732# | 80,960.23 ± 192#* | 121,498.34 ± 576#* |
| BSA + Fructose + KY 1.00 mg/ml | 26,610.42 ± 653#* | 50,846.87 ± 912# | 71,943.30 ± 232# | 85,986.22 ± 653#* |
| BSA + Fructose + KY 1.50 mg/ml | 19,264.21 ± 183# | 46,255.54 ± 832# | 71,565.67 ± 579#* | 65,281.40 ± 183# |
| BSA + Fructose + KY 2.00 mg/ml | 15,785.34 ± 124#* | 36,412.20 ± 230#* | 49,098.19 ± 480# | 58,517.12 ± 124#* |
| BSA + Fructose + AG 0.50 mg/ml | 39,130.21 ± 843# | 63,388.56 ± 153# | 114,182.12 ± 523# | 161,529.34 ± 843# |
| BSA + Fructose + AG 1.00 mg/ml | 12,807.29 ± 473 | 42,917.12 ± 322 | 62,121.87 ± 587 | 81,464.64 ± 473 |

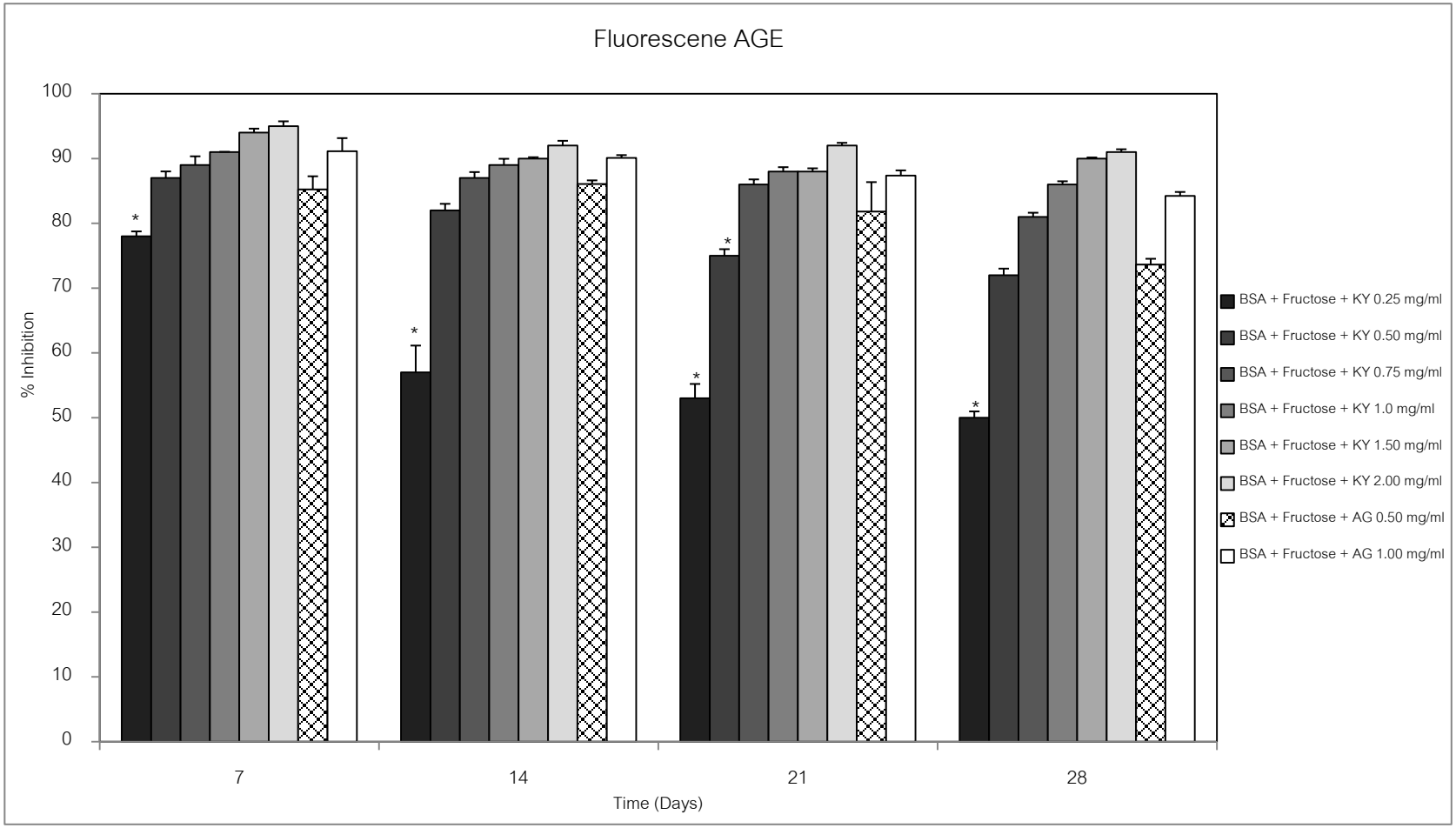
Data are expressed as mean ± SEM, n =3. #$P < 0.01$ compared to BSA+Fructose and * $P < 0.05$ compared to aminoguanidine 1.00 mg/ml.

**Table 15 –** The percentage inhibition of pomelo cultivar (Kao Yai) on the formation of fluorescence AGE at day 7, 14, 21 and 28.

| | % Inhibition | | | |
|---|---|---|---|---|
| | Day 7 | Day 14 | Day 21 | Day 28 |
| BSA + Fructose + KY 0.25 mg/ml | 78.37 ± 0.74* | 67.32 ± 4.12* | 59.05 ± 2.20* | 49.97 ± 0.96* |
| BSA + Fructose + KY 0.50 mg/ml | 87.49 ± 1.19 | 82.32 ± 1.37 | 74.69 ± 1.64 | 71.53 ± 0.52* |
| BSA + Fructose + KY 0.75 mg/ml | 88.68 ± 1.33 | 87.24 ± 0.90 | 86.38 ± 0.77 | 80.65 ± 0.63 |
| BSA + Fructose + KY 1.00 mg/ml | 91.04 ± 0.03 | 88.85 ± 0.95 | 87.01 ± 0.66 | 86.31 ± 0.48 |
| BSA + Fructose + KY 1.50 mg/ml | 93.51 ± 0.60 | 89.88 ± 0.19 | 87.97 ± 0.48 | 89.61 ± 0.15 |
| BSA + Fructose + KY 2.00 mg/ml | 94.68 ± 0.73 | 92.06 ± 0.73 | 91.75 ± 0.42 | 90.68 ± 0.42 |
| BSA + Fructose + AG 0.50 mg/ml | 85.23 ± 1.89 | 86.06 ± 0.56 | 81.81± 4.54 | 73.62 ± 0.90 |
| BSA + Fructose + AG 1.00 mg/ml | 91.13 ± 2.73 | 90.10 ± 0.42 | 87.36 ± 0.80 | 84.23 ± 0.60 |

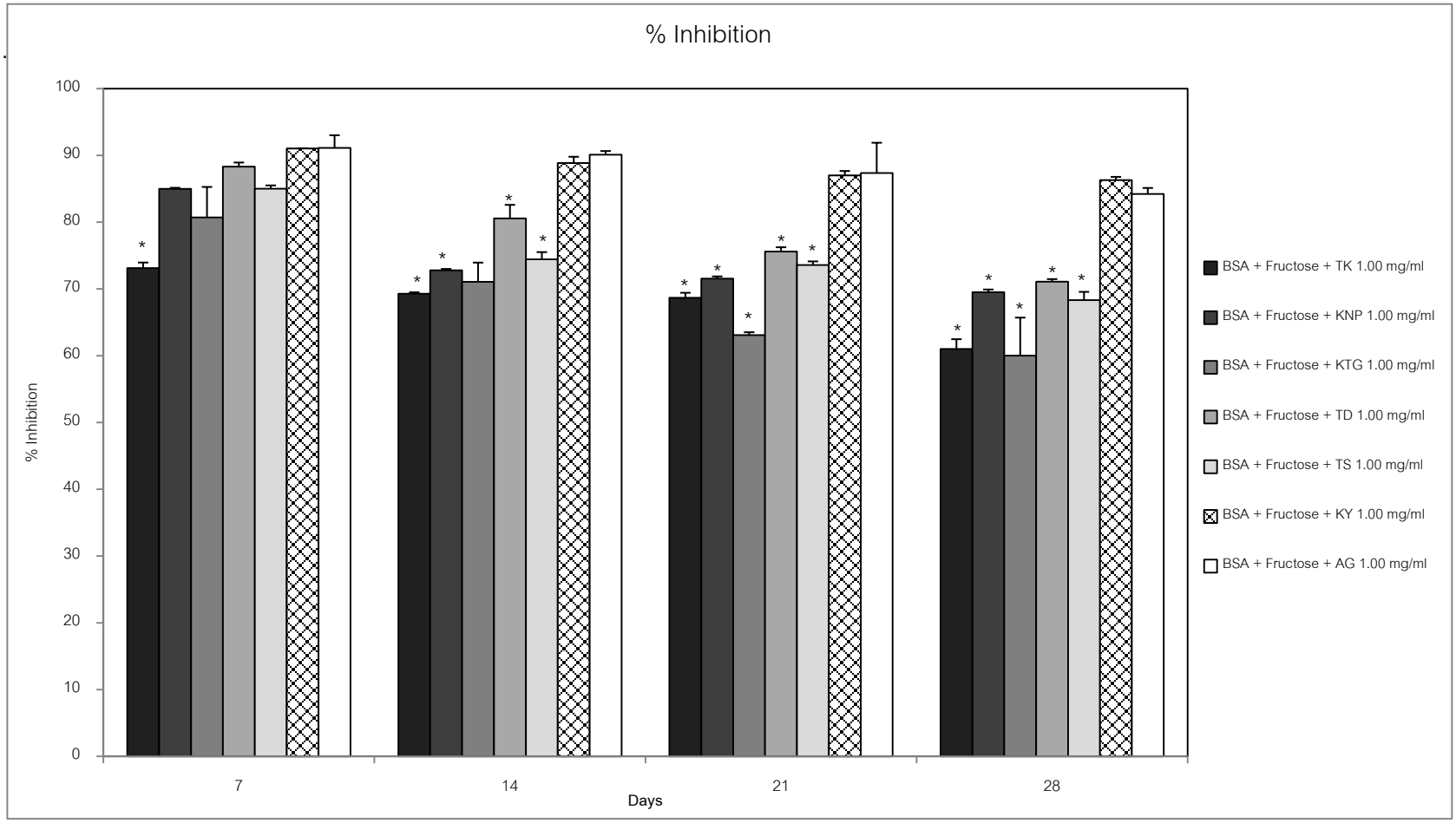
Data are expressed as mean ± SEM, n =3.*$P < 0.05$ compared to aminoguanidine 1.00 mg/ml.

**Figure 23** – The percentage inhibition of pomelo cultivar (Kao Yai) on the formation of fluorescence AGE at day 7, 14, 21 and 28




Data are expressed as mean ± SEM, n =3.*$P < 0.05$ compared to aminoguanidine 1.00 mg/ml.

**Figure 24** - The percentage inhibition of pomelo cultivars (1.00 mg/ml) on the formation of fluorescence AGE at day 7, 14, 21 and 28 compared to aminoguanidine (1.00 mg/ml)




Data are expressed as mean ± SEM, n =3.* $P < 0.05$ compared to aminoguanidine 1.00 mg/ml.

### 2.2. Determination of fructosamine

Table 16-21 shows the concentration of fructosamine of the six pomelo cultivars at day 7, 14, 21 and 28. An elevation of fructosamine was observed in the incubation of BSA + fructose (negative control). The results showed that the pomelo cultivar extracts markedly reduced the concentration of fructosamine in a concentration dependent manner.

At day 7, when compared to the BSA + fructose the pomelo cultivars Thong Dee, Kao Yai, Kao Nam Pueng, Tar Koi, Tubtim Siam and Kao Tanggwa at 1.00 mg/ml showed the ability to reduce the level of fructosamine by a 29.94±0.09, 27.68±0.01, 22.60±0.02, 21.47±0.08, 16.95±0.04, 8.47±0.01%, respectively. Additionally, when compared to aminoguanidine 1.00 mg/ml, the pomelo cultivars that were able to exert a higher reducing ability than aminoguanidine at the equivalent concentration were Thong Dee and Kao Yai which exerted a 5.34±0.01 and 2.29±0.09% respectively, higher. On the other hand, the other pomelo cultivars, Kao Nam Pueng, Tar Koi, Tubtim Siam and Kao Tanggwa at 1.00 mg/ml exerted a lesser amount of decrease ability than aminoguanidine (1.00 mg/ml) by 4.58±0.01, 6.11±0.02, 12.11±0.05 and 23.66±0.04%, respectively.

At day 14, when compared to the BSA + fructose the pomelo cultivars Thong Dee, Tar Koi, Kao Yai, Tubtim Siam, Kao Nam Pueng and Kao Tanggwa at 1.00 mg/ml exhibited the ability to reduce the level of fructosamine by 34.10±0.03, 34.10±0.02, 33.64±0.01, 27.65±0.01, 27.19±0.09 and 22.12±0.04%, respectively. Moreover, when compared to aminoguanidine 1.00 mg/ml, the pomleo cultivars that were able to exert a higher reducing ability than aminoguanidine at the equivalent concentration was Thong Dee, Tar Koi, Kao Yai, Tubtim Siam and Kao Nam Pueng that exerted a 10.06±0.08, 10.06±0.01, 9.43±0.04, 1.26±0.04 and 0.63±0.09% higher in reduction, respectively. The Kao Tanggwa pomelo cultivar at 1.00 mg/ml when compared to the

aminoguanidine (1.00 mg.ml), however, exerted a lesser reducing ability by 6.29±0.07%.

At day 21, when compared to the BSA + fructose the pomelo cultivars Thong Dee, Kao Nam Pueng, Tubtim Siam, Tar Koi, Kao Tanggwa and Kao Yai at 1.00 mg/ml was able to reduce the level of fructosamine by 33.33±0.03, 28.92±0.04, 28.92±0.01, 27.31±0.03, 25.30±0.08 and 24.50±0.05%, respectively. In addition, when compared to aminoguanidine 1.00 mg/ml, the only pomleo cultivars that were able to exert a higher reducing ability than aminoguanidine at the equivalent concentration were Thong Dee, and Kao Nam Pueng with a 5.68±0.01 and 3.98±0.02% higher, respectively. In contrast, the pomelo cultivars Tubtim Siam, Tar Koi, Kao Tanggwa and Kao Yai at 1.00 mg/ml, exerted a lesser ability of reduction as compared to aminoguanidine (1.00 mg/ml) by 0.57±0.01, 2.84±0.02, 5.68±0.09 and 6.82±0.04%, respectively.

At day 28, the pomelo cultivars Thong Dee, Tar Koi, Kao Tanggwa, Tubtim Siam, Kao Yai and Kao Nam Pueng at 1.00 mg/ml exerted a 32.60±0.04, 30.40±0.03, 25.64±0.01, 23.08±0.02, 17.58±0.04 and 16.48±0.03% higher, respectively. When compared to aminoguanidine 1.00 mg/ml, the only pomleo cultivars that were able to exert a higher reducing ability than aminoguanidine at the equivalent concentration were Thong Dee, Tar Koi and Tubtim Siam that showed a 9.80±0.01, 6.86±0.07, 0.49±0.01% higher. Alternatively, the pomelo cultivars Kao Tanggwa, Kao Yai and Kao Nam Pueng at 1.00 mg/ml exerted a lesser ability of reduction as compared to aminoguanidine (1.00 mg/ml) by 0.57±0.01, 2.84±0.02, 5.68±0.09 and 6.82±0.04%, respectively.

**Table 16 –** The effect of the pomelo cultivar (Tar Koi) on the formations of fructosamine at day 7, 14, 21 and 28.

| | Fructosamine (mM) | | | |
|---|---|---|---|---|
| | Day 7 | Day 14 | Day 21 | Day 28 |
| BSA | 1.03 ± 0.02 | 1.12 ± 0.06 | 1.23 ± 0.20 | 1.49 ± 0.08 |
| BSA + Fructose | 1.77 ± 0.07 | 2.17 ± 0.06 | 2.49 ± 0.16 | 2.73 ± 0.33 |
| BSA + Fructose + TK 0.25 mg/ml | 1.69 ± 0.43 | 1.96 ± 0.11 | 2.22 ± 0.04 | 2.37 ± 0.20* |
| BSA + Fructose + TK 0.50 mg/ml | 1.51 ± 0.28 | 1.73 ± 0.11 | 2.10 ± 0.20 | 2.14 ± 0.10# |
| BSA + Fructose + TK 0.75 mg/ml | 1.43 ± 0.20# | 1.62 ± 0.11# | 1.92 ± 0.04 | 2.03 ± 0.10# |
| BSA + Fructose + TK 1.00 mg/ml | 1.39 ± 0.07# | 1.43 ± 0.26# | 1.81 ± 0.23# | 1.90 ± 0.09# |
| BSA + Fructose + TK 1.50 mg/ml | 1.28 ± 0.11# | 1.32 ± 0.10# | 1.69 ± 0.26# | 1.88 ± 0.14# |
| BSA + Fructose + TK 2.00 mg/ml | 1.13 ± 0.10# | 1.24 ± 0.04#* | 1.54 ± 0.75# | 1.77 ± 0.15# |
| BSA + Fructose + AG 0.50 mg/ml | 1.47 ± 0.21# | 1.88 ± 0.16# | 1.88 ± 0.06# | 2.39 ± 0.01# |
| BSA + Fructose + AG 1.00 mg/ml | 1.31 ± 0.04# | 1.59 ± 0.21# | 1.76 ± 0.60# | 2.04 ± 0.02# |

Data are expressed as mean ± SEM, n =3. #$P < 0.05$ compared to BSA+Fructose and *$P < 0.05$ compared to aminoguanidine 1.00 mg/ml.

**Table 17 –** The effect of the pomelo cultivar (Kao Nam Pueng) on the formations of fructosamine at day 7, 14, 21 and 28.

| | Fructosamine (mM) | | | |
|---|---|---|---|---|
| | Day 7 | Day 14 | Day 21 | Day 28 |
| BSA | 1.03 ± 0.02 | 1.12 ± 0.06 | 1.23 ± 0.20 | 1.49 ± 0.08 |
| BSA + Fructose | 1.77 ± 0.07 | 2.17 ± 0.06 | 2.49 ± 0.16 | 2.73 ± 0.33 |
| BSA + Fructose + KNP 0.25 mg/ml | 1.58 ± 0.19* | 1.96 ± 0.05* | 2.04 ± 0.05 | 2.82 ± 0.19# |
| BSA + Fructose + KNP 0.50 mg/ml | 1.53 ± 0.11* | 1.74 ± 0.19#* | 1.99 ± 0.28* | 2.73 ± 0.23# |
| BSA + Fructose + KNP 0.75 mg/ml | 1.41 ± 0.09# | 1.63 ± 0.14# | 1.89 ± 0.19# | 2.36 ± 0.53# |
| BSA + Fructose + KNP 1.00 mg/ml | 1.37 ± 0.11# | 1.58 ± 0.16# | 1.79 ± 0.09# | 2.28 ± 0.15# |
| BSA + Fructose + KNP 1.50 mg/ml | 1.31 ± 0.05# | 1.53 ± 1.19# | 1.62 ± 0.30# | 2.01 ± 0.09# |
| BSA + Fructose + KNP 2.00 mg/ml | 1.26 ± 0.01# | 1.42 ± 0.09# | 1.54 ± 0.30# | 1.85 ± 0.46# |
| BSA + Fructose + AG 0.50 mg/ml | 1.47 ± 0.21# | 1.88 ± 0.16# | 1.88 ± 0.06# | 2.39 ± 0.01# |
| BSA + Fructose + AG 1.00 mg/ml | 1.31 ± 0.04# | 1.59 ± 0.21# | 1.76 ± 0.60# | 2.04 ± 0.02# |

Data are expressed as mean ± SEM, n =3. #$P < 0.05$ compared to BSA+Fructose and *$P < 0.05$ compared to aminoguanidine 1.00 mg/ml.

**Table 18 –** The effect of the pomelo cultivar (Kao Tanggwa) on the formations of fructosamine at day 7, 14, 21 and 28.

| | Fructosamine (mM) | | | |
|---|---|---|---|---|
| | Day 7 | Day 14 | Day 21 | Day 28 |
| BSA | 1.03 ± 0.02 | 1.12 ± 0.06 | 1.23 ± 0.20 | 1.49 ± 0.08 |
| BSA + Fructose | 1.77 ± 0.07 | 2.17 ± 0.06 | 2.49 ± 0.16 | 2.73 ± 0.33 |
| BSA + Fructose + KTG 0.25 mg/ml | 1.88 ± 0.14# | 2.01 ± 0.17# | 2.11 ± 0.23 | 2.52 ± 0.17 |
| BSA + Fructose + KTG 0.50 mg/ml | 1.81 ± 0.14 | 1.94 ± 0.29# | 2.02 ± 0.17# | 2.29 ± 0.19# |
| BSA + Fructose + KTG 0.75 mg/ml | 1.77 ± 0.25 | 1.86 ± 0.14# | 1.99 ± 0.11# | 2.18 ± 0.11# |
| BSA + Fructose + KTG 1.00 mg/ml | 1.62 ± 0.11# | 1.69 ± 0.23# | 1.86 ± 0.17# | 2.10 ± 0.36# |
| BSA + Fructose + KTG 1.50 mg/ml | 1.36 ± 0.07# | 1.59 ± 0.14# | 1.73 ± 0.17# | 1.84 ± 0.22#* |
| BSA + Fructose + KTG 2.00 mg/ml | 1.28 ± 0.06# | 1.48 ± 0.16# | 1.66 ± 0.55# | 1.77 ± 0.04# |
| BSA + Fructose + AG 0.50 mg/ml | 1.47 ± 0.21# | 1.88 ± 0.16# | 1.88 ± 0.06# | 2.39 ± 0.01# |
| BSA + Fructose + AG 1.00 mg/ml | 1.31 ± 0.04# | 1.59 ± 0.21# | 1.76 ± 0.60# | 2.04 ± 0.02# |

Data are expressed as mean ± SEM, n =3. #$P < 0.05$ compared to BSA+Fructose and *$P < 0.05$ compared to aminoguanidine 1.00 mg/ml.

**Table 19 –** The effect of the pomelo cultivar (Thong Dee) on the formations of fructosamine at day 7, 14, 21 and 28.

| | Fructosamine (mM) | | | |
|---|---|---|---|---|
| | Day 7 | Day 14 | Day 21 | Day 28 |
| BSA | 1.03 ± 0.02 | 1.12 ± 0.06 | 1.23 ± 0.20 | 1.49 ± 0.08 |
| BSA + Fructose | 1.77 ± 0.07 | 2.17 ± 0.06 | 2.49 ± 0.16 | 2.73 ± 0.33 |
| BSA + Fructose + TD 0.25 mg/ml | 1.66 ± 0.21 | 1.88 ± 0.14 | 1.99 ± 0.39$^{\#}$ | 2.22 ± 0.27$^{\#}$ |
| BSA + Fructose + TD 0.50 mg/ml | 1.51 ± 0.06 | 1.69 ± 0.20$^{\#}$ | 1.84 ± 0.74$^{\#}$ | 2.07 ± 0.06$^{\#}$ |
| BSA + Fructose + TD 0.75 mg/ml | 1.32 ± 0.21$^{\#}$ | 1.51 ± 0.06$^{\#}$ | 1.77 ± 0.75$^{\#}$ | 1.96 ± 0.17$^{\#}$ |
| BSA + Fructose + TD 1.00 mg/ml | 1.24 ± 0.07$^{\#}$ | 1.43 ± 0.32$^{\#}$ | 1.66 ± 0.23$^{\#}$ | 1.84 ± 0.28$^{\#}$ |
| BSA + Fructose + TD 1.50 mg/ml | 1.13 ± 0.20$^{\#}$ | 1.36 ± 0.14$^{\#}$ | 1.54 ± 0.51$^{\#*}$ | 1.77 ± 0.07$^{\#}$ |
| BSA + Fructose + TD 2.00 mg/ml | 1.06 ± 0.11$^{\#*}$ | 1.24 ± 0.16$^{\#}$ | 1.47 ± 0.17$^{\#}$ | 1.62 ± 0.30$^{\#}$ |
| BSA + Fructose + AG 0.50 mg/ml | 1.47 ± 0.21$^{\#}$ | 1.88 ± 0.16$^{\#}$ | 1.88 ± 0.06$^{\#}$ | 2.39 ± 0.01$^{\#}$ |
| BSA + Fructose + AG 1.00 mg/ml | 1.31 ± 0.04$^{\#}$ | 1.59 ± 0.21$^{\#}$ | 1.76 ± 0.60$^{\#}$ | 2.04 ± 0.02$^{\#}$ |

Data are expressed as mean ± SEM, n =3. $^{\#}P < 0.05$ compared to BSA+Fructose and $^{*}P < 0.05$ compared to aminoguanidine 1.00 mg/ml.

**Table 20 –** The effect of the pomelo cultivar (Tubtim Siam) on the formations of fructosamine at day 7, 14, 21 and 28.

| | Fructosamine (mM) | | | |
|---|---|---|---|---|
| | Day 7 | Day 14 | Day 21 | Day 28 |
| BSA | 1.03 ± 0.02 | 1.12 ± 0.06 | 1.23 ± 0.20 | 1.49 ± 0.08 |
| BSA + Fructose | 1.77 ± 0.07 | 2.17 ± 0.06 | 2.49 ± 0.16 | 2.73 ± 0.33 |
| BSA + Fructose + TS 0.25 mg/ml | 1.77 ± 0.04 | 1.92 ± 0.31 | 2.18 ± 0.11 | 2.55 ± 0.16 |
| BSA + Fructose + TS 0.50 mg/ml | 1.69 ± 0.10 | 1.75 ± 0.07$^{\#}$ | 1.96 ± 0.06$^{\#}$ | 2.18 ± 0.28$^{\#}$ |
| BSA + Fructose + TS 0.75 mg/ml | 1.58 ± 0.07$^{\#}$ | 1.69 ± 0.10$^{\#}$ | 1.88 ± 0.04$^{\#}$ | 2.10 ± 0.04$^{\#}$ |
| BSA + Fructose + TS 1.00 mg/ml | 1.47 ± 0.04$^{\#}$ | 1.57 ± 0.15$^{\#}$ | 1.77 ± 0.04$^{\#}$ | 2.03 ± 0.04$^{\#}$ |
| BSA + Fructose + TS 1.50 mg/ml | 1.32 ± 0.31$^{\#}$ | 1.32 ± 0.10$^{\#*}$ | 1.62 ± 0.17$^{\#}$ | 1.92 ± 0.10$^{\#*}$ |
| BSA + Fructose + TS 2.00 mg/ml | 1.21 ± 0.16$^{\#}$ | 1.28 ± 0.13$^{\#*}$ | 1.54 ± 0.10$^{\#}$ | 1.84 ± 0.06$^{\#}$ |
| BSA + Fructose + AG 0.50 mg/ml | 1.47 ± 0.21$^{\#}$ | 1.88 ± 0.16$^{\#}$ | 1.88 ± 0.06$^{\#}$ | 2.39 ± 0.01$^{\#}$ |
| BSA + Fructose + AG 1.00 mg/ml | 1.31 ± 0.04$^{\#}$ | 1.59 ± 0.21$^{\#}$ | 1.76 ± 0.60$^{\#}$ | 2.04 ± 0.02$^{\#}$ |

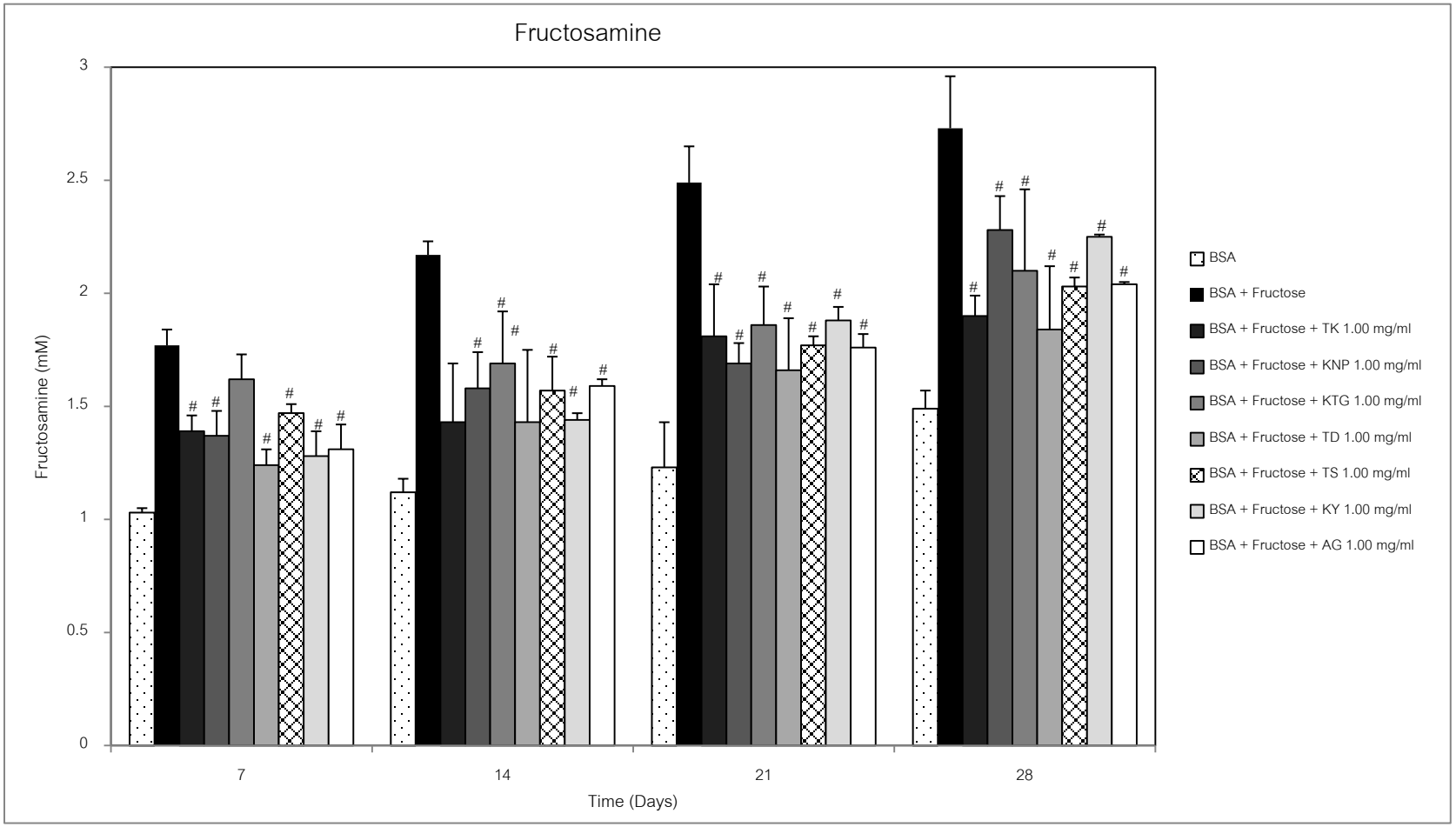
Data are expressed as mean ± SEM, n =3. $^{\#}P < 0.05$ compared to BSA+Fructose and $^{*}P < 0.05$ compared to aminoguanidine 1.00 mg/ml.

**Table 21 –** The effect of the pomelo cultivar (Kao Yai) on the formations of fructosamine at day 7, 14, 21 and 28.

| | Fructosamine (mM) | | | |
|---|---|---|---|---|
| | Day 7 | Day 14 | Day 21 | Day 28 |
| BSA | 1.03 ± 0.02 | 1.12 ± 0.06 | 1.23 ± 0.20 | 1.49 ± 0.08 |
| BSA + Fructose | 1.77 ± 0.07 | 2.17 ± 0.06 | 2.49 ± 0.16 | 2.73 ± 0.33 |
| BSA + Fructose + KY 0.25 mg/ml | 1.47 ± 0.21 | 1.80 ± 0.04$^{\#}$ | 2.31 ± 0.01 | 2.63± 0.02 |
| BSA + Fructose + KY 0.50 mg/ml | 1.39 ± 0.06$^{\#}$ | 1.70 ± 0.03$^{\#}$ | 2.07 ± 0.12$^{\#}$ | 2.46 ± 0.02 |
| BSA + Fructose + KY 0.75 mg/ml | 1.32 ± 0.07$^{\#}$ | 1.57 ± 0.01$^{\#}$ | 2.01 ± 0.31$^{\#}$ | 2.39 ± 0.01$^{\#}$ |
| BSA + Fructose + KY 1.00 mg/ml | 1.28 ± 0.11$^{\#}$ | 1.44 ± 0.03$^{\#}$ | 1.88 ± 0.06$^{\#}$ | 2.25 ± 0.01$^{\#}$ |
| BSA + Fructose + KY 1.50 mg/ml | 1.21 ± 0.07$^{\#}$ | 1.39 ± 0.01$^{\#}$ | 1.70 ± 0.16$^{\#}$ | 2.13 ± 0.01$^{\#}$ |
| BSA + Fructose + KY 2.00 mg/ml | 1.13 ± 0.10$^{*\#}$ | 1.20 ± 0.01$^{\#}$ | 1.57 ± 0.21$^{\#}$ | 2.03 ± 0.03$^{\#}$ |
| BSA + Fructose + AG 0.50 mg/ml | 1.47 ± 0.21$^{\#}$ | 1.88 ± 0.16$^{\#}$ | 1.88 ± 0.06$^{\#}$ | 2.39 ± 0.01$^{\#}$ |
| BSA + Fructose + AG 1.00 mg/ml | 1.31 ± 0.04$^{\#}$ | 1.59 ± 0.21$^{\#}$ | 1.76 ± 0.60$^{\#}$ | 2.04 ± 0.02$^{\#}$ |

Data are expressed as mean ± SEM, n =3. $^{\#}P < 0.05$ compared to BSA+Fructose and $^{*}P < 0.05$ compared to aminoguanidine 1.00 mg/ml.

**Figure 24 –** The effect of the pomelo cultivars (1.00 mg/ml) on the formations of fructosamine at day 7, 14, 21 and 28 compared to aminoguanidine 1.00 mg/ml




Data are expressed as mean ± SEM, n =3. $^{\#}P < 0.05$ compared to BSA+Fructose and $^{*}P < 0.05$ compared to aminoguanidine 1.00 mg/ml.

## 2.3. Evaluation of protein oxidation

### 2.3.1. Formation of protein carbonyl content

The effects of pomelo cultivar extracts on the protein carbonyl content induce by protein oxidation derived from glycation are shown in Table 22 - 27. An increase in protein carbonyl content was observed in BSA + fructose (negative control) during the experiment period. The results demonstrated that the pomelo cultivar extract reduced the protein carbonyl content in a concentration-dependent manner.

In general, all concentrations (0.25, 0.50, 0.75, 1.0, 1.5 and 2.00 mg/ml) of all six pomelo cultivars showed a significant decrease in the formation of protein carbonyl content when compared to the BSA + fructose ($P<0.01$) and the highest amount of inhibition was noticed at the concentration of 2.00 mg/ml. At day 7, the pomelo cultivars Thong Dee, Tar Koi, Kao Yai, Kao Nam Pueng, Tubtim Siam and Kao Tanggwa at 1.00 mg/ml showed the ability to reduce the amount of formed protein carbonyl content by 62.29±0.01, 54.00±0.02, 52.70±0.01, 50.18±0.04, 50.06±0.04 and 44.66±0.02% when compared to the BSA + fructose, respectively. Moreover, when compared to the amount exerted by the aminoguanidine (1.00 mg/ml), only the Thong Dee, Tar Koi, Kao Yai pomelo cultivar at 1.00 mg/ml were able to exert a 21.88±0.08, 4.83±0.03 and 2.04±0.01% higher in the reduction of protein carbonyl content, respectively. Conversely, the Kao Nam Pueng, Tubtim Siam and Kao Tanggwa pomelo cultivar at 1.00 mg/ml showed a lesser amount of reduction ability by 3.18±0.02, 3.44±0.03 and 14.63±0.05% than that of aminoguanidine (1.00 mg/ml), respectively.

At day 14, the pomelo cultivars Kao Yai, Kao Nam Pueng, Thong Dee, Tar Koi, Kao Tanggwa and Tubtim Siam at the concentration of 1.00 mg/ml showed a 60.72±0.03, 52.67±0.02, 34.17±0.05, 30±0.01, 24.37±0.02 and 11.31±0.08% in the reduction in protein carbonyl content when compared to the BSA + fructose, respectively. In addition, when compared to the amount reduced carbonyl content exhibited by aminoguanidine (1.00 mg/ml), the Kao Yai, Kao Nam Pueng and Thong Dee pomelo cultivar at the same concentration, exhibited a 42.68±0.08, 17.13±0.08

and 3.98±0.08% higher reduction, respectively. As for the Tar Koi, Kao Tanggwa and Tubtim Siam pomelo cultivar at 1.00 mg/ml showed a 0.48±0.01, 10.36±0.05 and 29.42±0.03% lesser amount than that of exerted by aminoguanidine (1.00 mg/ml), respectively.

At day 21, only pomelo cultivars Kao Yai, Kao Nam Pueng, Tar Koi and Kao Tanggwa at 1.00 mg/ml was able to show a 45.42±0.02, 35.88±0.04, 30±0.03 and 27.40±0.01% of reduction in protein carbonyl content when compared to the BSA + fructose, respectively. Additionally, the Kao Yai pomleo cultivar was the only pomelo cultivar that was able to exert a 7.78±0.09% higher amount of reduction in protein carbonyl content than aminoguanidine (1.00mg/ml). While the Kao Nam Pueng, Tar Koi, Kao Tanggwa, Thong Dee and Tubtim Siam pomelo cultivar at 1.00 mg/ml exerted a 7.70±10.01, 17.47±0.03, 18.49±0.05, 29.06±0.08 and 43.81±0.02% lesser amount than that was exerted by the aminoguanidine (1.00 mg/ml), respectively.

At day 28, in a similar manner, only pomelo cultivars that were able to show a decrease in the protein carbonyl content was the Kao Nam Pueng, Tar Koi, Kao Yai and Kao Tanggwa at 1.00 mg/ml that exerted a 26.45±0.01, 20.79±0.01, 9.21±0.09 and 6.35±0.02% of decrease when compared to the BSA + Fructose, respectively. Moreover, at day 28, the only pomelo cultivar at 1.00 mg/ml that was able to exert a higher reducing ability in the protein carbonyl content than aminoguanidine at 1.00 mg/ml was Kao Nam Pueng that exerted a 7.00±0.01% higher. The Tar Koi, Kao Yai, Thong Dee, Kao Tanggwa and Tubtim Siam pomelo cultivar at 1.00 mg/ml conversely exerted a 0.58±0.01, 13.29±0.01, 13.72±0.01, 15.93±0.01 and 30.76±0.01% lesser amount in the reduction of the protein carbonyl content than aminoguanidine (1.00 mg/ml), respectively.

**Table 22** - The effect of the pomelo cultivar (Tar Koi) on the formations of protein carbonyl content at day 7, 14, 21 and 28.

| Carbonyl content (nmol carbonyl/mg protein) | | | | |
|---|---|---|---|---|
| | Day 7 | Day 14 | Day 21 | Day 28 |
| BSA | 3.02 ± 0.10 | 5.21 ± 0.03 | 8.39 ± 0.01 | 10.04 ± 0.10 |
| BSA + Fructose | 16.28 ± 0.88 | 21.13 ± 1.05 | 23.47 ± 1.27 | 26.28 ± 3.22 |
| BSA + Fructose + TK 0.25 mg/ml | 8.81 ± 0.39$^{\#}$ | 18.94 ± 0.42 | 21.85 ± 1.59 | 24.01 ± 1.24 |
| BSA + Fructose + TK 0.50 mg/ml | 8.46 ± 0.77$^{\#}$ | 16.90 ± 1.48 | 19.17 ± 1.69 | 23.86 ± 0.09 |
| BSA + Fructose + TK 0.75 mg/ml | 8.09 ± 0.40$^{\#}$ | 15.79 ± 1.62$^{\#}$ | 18.23 ± 1.13 | 22.99 ± 2.40 |
| BSA + Fructose + TK 1.00 mg/ml | 7.48 ± 0.84$^{\#}$ | 14.55 ± 1.18$^{\#}$ | 16.81 ± 1.33$^{\#}$ | 20.81 ± 2.62 |
| BSA + Fructose + TK 1.50 mg/ml | 7.03 ± 0.19$^{\#}$ | 13.30 ± 0.16$^{\#}$ | 15.98 ± 2.41$^{\#}$ | 17.53 ± 0.54$^{\#}$ |
| BSA + Fructose + TK 2.00 mg/ml | 6.83 ± 3.25$^{\#}$ | 12.54 ± 1.24$^{\#}$ | 14.95 ± 0.75$^{\#*}$ | 16.67 ± 1.08$^{\#}$ |
| BSA + Fructose + AG 0.50 mg/ml | 8.25 ± 1.11$^{\#}$ | 16.44 ± 0.71$^{\#}$ | 15.11 ± 0.56$^{\#}$ | 23.34 ± 3.48 |
| BSA + Fructose + AG 1.00 mg/ml | 7.86 ± 0.88$^{\#}$ | 14.48 ± 2.29$^{\#}$ | 16.89 ± 2.46$^{\#}$ | 20.69 ± 2.24$^{\#}$ |

Data are expressed as mean ± SEM, n =3. $^{\#}P < 0.05$ compared to BSA+Fructose and $^{*}P < 0.05$ compared to aminoguanidine 1.00 mg/ml.

**Table 23** - The effect of the pomelo cultivar (Kao Nam Pueng) on the formations of protein carbonyl content at day 7, 14, 21 and 28.

| | Carbonyl content (nmol carbonyl/mg protein) | | | |
|---|---|---|---|---|
| | Day 7 | Day 14 | Day 21 | Day 28 |
| BSA | 3.02 ± 0.10 | 5.21 ± 0.03 | 8.39 ± 0.01 | 10.04 ± 0.10 |
| BSA + Fructose | 16.28 ± 0.88 | 21.13 ± 1.05 | 23.47 ± 1.27 | 26.28 ± 3.22 |
| BSA + Fructose + KNP 0.25 mg/ml | 10.11 ± 1.18$^{\#}$ | 13.03 ± 1.47$^{\#}$ | 17.97 ± 1.21 | 25.96 ± 2.52 |
| BSA + Fructose + KNP 0.50 mg/ml | 9.40 ± 1.77$^{\#}$ | 12.24 ± 0.27$^{\#}$* | 16.79 ± 1.07 | 23.99 ± 2.59 |
| BSA + Fructose + KNP 0.75 mg/ml | 9.12 ± 1.03$^{\#}$ | 10.59 ± 0.85$^{\#}$ | 16.55 ± 0.55 | 20.75 ± 1.37 |
| BSA + Fructose + KNP 1.00 mg/ml | 8.11 ± 0.54$^{\#}$ | 12.00 ± 0.76$^{\#}$ | 15.05 ± 2.28$^{\#}$ | 19.33 ± 3.59$^{\#}$ |
| BSA + Fructose + KNP 1.50 mg/ml | 7.46 ± 2.11$^{\#}$ | 9.26 ± 1.99$^{\#}$ | 13.96 ± 1.07$^{\#}$ | 17.43 ± 2.26 |
| BSA + Fructose + KNP 2.00 mg/ml | 7.11 ± 0.94$^{\#}$ | 8.30 ± 1.80$^{\#}$* | 11.78 ± 1.07$^{\#}$ | 16.27 ± 3.70$^{\#}$ |
| BSA + Fructose + AG 0.50 mg/ml | 8.25 ± 1.11$^{\#}$ | 16.44 ± 0.71$^{\#}$ | 15.11 ± 0.56$^{\#}$ | 23.34 ± 3.48 |
| BSA + Fructose + AG 1.00 mg/ml | 7.86 ± 0.88$^{\#}$ | 14.48 ± 2.29$^{\#}$ | 16.89 ± 2.46$^{\#}$ | 20.69 ± 2.24$^{\#}$ |

Data are expressed as mean ± SEM, n =3. $^{\#}P < 0.05$ compared to BSA+Fructose and $^{*}P < 0.05$ compared to aminoguanidine 1.00 mg/ml.

**Table 24** - The effect of the pomelo cultivar (Kao Tanggwa) on the formations of protein carbonyl content at day 7, 14, 21 and 28.

| | Carbonyl content (nmol carbonyl/mg protein) | | | |
|---|---|---|---|---|
| | Day 7 | Day 14 | Day 21 | Day 28 |
| BSA | 3.02 ± 0.10 | 5.21 ± 0.03 | 8.39 ± 0.01 | 10.04 ± 0.10 |
| BSA + Fructose | 16.28 ± 0.88 | 21.13 ± 1.05 | 23.47 ± 1.27 | 26.28 ± 3.22 |
| BSA + Fructose + KTG 0.25 mg/ml | 11.73 ± 3.91# | 21.37 ± 0.39 | 20.06 ± 2.20 | 31.95 ± 2.24 |
| BSA + Fructose + KTG 0.50 mg/ml | 11.45 ± 1.18# | 19.49 ± 1.58 | 19.12 ± 0.38 | 30.71 ± 1.43 |
| BSA + Fructose + KTG 0.75 mg/ml | 10.72 ± 1.59# | 17.87 ± 0.51 | 17.93 ± 3.14 | 28.20 ± 1.90 |
| BSA + Fructose + KTG 1.00 mg/ml | 9.01 ± 0.43# | 15.98 ± 1.28# | 17.04 ± 2.20# | 24.61 ± 0.84 |
| BSA + Fructose + KTG 1.50 mg/ml | 8.71 ± 1.56# | 14.80 ± 1.63# | 16.64 ± 1.07 | 22.83 ± 1.76 |
| BSA + Fructose + KTG 2.00 mg/ml | 7.79 ±.122# | 13.72 ± 0.99# | 15.83 ± 0.42# | 20.82 ± 1.47 |
| BSA + Fructose + AG 0.50 mg/ml | 8.25 ± 1.11# | 16.44 ± 0.71# | 15.11 ± 0.56# | 23.34 ± 3.48 |
| BSA + Fructose + AG 1.00 mg/ml | 7.86 ± 0.88# | 14.48 ± 2.29# | 16.89 ± 2.46# | 20.69 ± 2.24# |

Data are expressed as mean ± SEM, n =3. #$P < 0.05$ compared to BSA+Fructose and * $P < 0.05$ compared to aminoguanidine 1.00 mg/ml.

**Table 25** - The effect of the pomelo cultivar (Thong Dee) on the formations of protein carbonyl content at day 7, 14, 21 and 28.

| | Carbonyl content (nmol carbonyl/mg protein) | | | |
|---|---|---|---|---|
| | Day 7 | Day 14 | Day 21 | Day 28 |
| BSA | 3.02 ± 0.10 | 5.21 ± 0.03 | 8.39 ± 0.01 | 10.04 ± 0.10 |
| BSA + Fructose | 16.28 ± 0.88 | 21.13 ± 1.05 | 23.47 ± 1.27 | 26.28 ± 3.22 |
| BSA + Fructose + TD 0.25 mg/ml | 7.99 ± 0.58 | 16.91 ± 0.40 | 26.13 ± 3.08 | 31.44 ± 3.42 |
| BSA + Fructose + TD 0.50 mg/ml | 7.43 ± 0.32$^{\#}$ | 15.96 ± 1.02 | 25.80 ± 2.01 | 30.13 ± 1.64 |
| BSA + Fructose + TD 0.75 mg/ml | 7.07 ± 0.56$^{\#}$ | 14.02 ± 1.45 | 24.31 ± 1.66 | 27.48 ± 1.65 |
| BSA + Fructose + TD 1.00 mg/ml | 6.14 ± 0.85$^{\#}$ | 13.91 ± 1.51$^{\#}$ | 19.58 ± 1.88 | 22.98 ± 2.81 |
| BSA + Fructose + TD 1.50 mg/ml | 5.51 ± 0.52$^{\#}$ | 12.54 ± 1.78$^{\#}$ | 21.20 ± 2.18 | 24.43 ± 1.21 |
| BSA + Fructose + TD 2.00 mg/ml | 4.75 ± 0.94$^{\#}$* | 11.90 ± 1.23$^{\#}$ | 16.89 ± 2.67 | 20.36 ± 1.92 |
| BSA + Fructose + AG 0.50 mg/ml | 8.25 ± 1.11$^{\#}$ | 16.44 ± 0.71$^{\#}$ | 15.11 ± 0.56 | 23.34 ± 3.48 |
| BSA + Fructose + AG 1.00 mg/ml | 7.86 ± 0.88$^{\#}$ | 14.48 ± 2.29$^{\#}$ | 16.89 ± 2.46$^{\#}$ | 20.69 ± 2.24$^{\#}$ |

Data are expressed as mean ± SEM, n =3. $^{\#}P < 0.05$ compared to BSA+Fructose and * $P < 0.05$ compared to aminoguanidine 1.00 mg/ml.

**Table 26** - The effect of the pomelo cultivar (Tubtim Siam) on the formations of protein carbonyl content at day 7, 14, 21 and 28.

| Carbonyl content (nmol carbonyl/mg protein) | | | | |
|---|---|---|---|---|
| | Day 7 | Day 14 | Day 21 | Day 28 |
| BSA | 3.02 ± 0.10 | 5.21 ± 0.03 | 8.39 ± 0.01 | 10.04 ± 0.10 |
| BSA + Fructose | 16.28 ± 0.88 | 21.13 ± 1.05 | 23.47 ± 1.27 | 26.28 ± 3.22 |
| BSA + Fructose + TS 0.25 mg/ml | 8.58 ± 0.34# | 19.92± 0.94 | 29.47 ± 2.17 | 35.15 ± 1.34 |
| BSA + Fructose + TS 0.50 mg/ml | 8.36 ± 0.14# | 19.51 ± 0.37 | 27.32 ± 0.97 | 34.64 ± 3.43 |
| BSA + Fructose + TS 0.75 mg/ml | 8.36 ± 0.14# | 19.00 ± 3.28 | 26.26 ± 0.03 | 32.25 ± 1.95 |
| BSA + Fructose + TS 1.00 mg/ml | 8.13 ± 0.41# | 18.74 ± 0.33 | 24.72 ± 2.22 | 29.88 ± 0.52 |
| BSA + Fructose + TS 1.50 mg/ml | 7.56 ± 0.79# | 18.12 ± 0.99 | 22.62 ± 1.22 | 27.02 ± 0.84 |
| BSA + Fructose + TS 2.00 mg/ml | 7.24 ± 0.52# | 17.25 ± 1.13 | 21.04 ± 0.75 | 24.51 ± 0.45 |
| BSA + Fructose + AG 0.50 mg/ml | 8.25 ± 1.11# | 16.44 ± 0.71# | 15.11 ± 0.56# | 23.34 ± 3.48 |
| BSA + Fructose + AG 1.00 mg/ml | 7.86 ± 0.88# | 14.48 ± 2.29# | 16.89 ± 2.46# | 20.69 ± 2.24# |

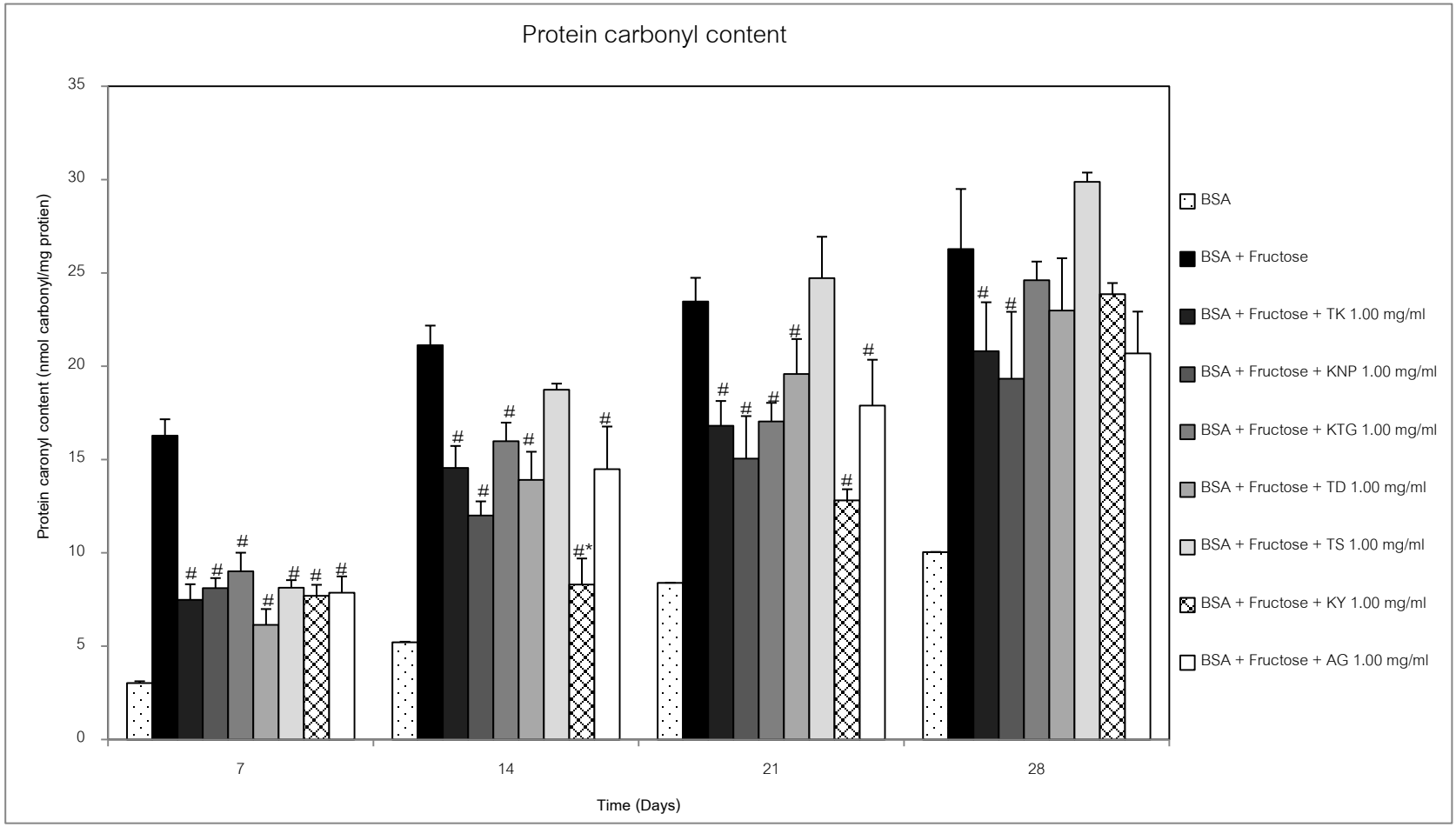
Data are expressed as mean ± SEM, n =3. #$P < 0.05$ compared to BSA+Fructose and *$P < 0.05$ compared to aminoguanidine 1.00 mg/ml.

**Table 27** - The effect of the pomelo cultivar (Kao Yai) on the formations of protein carbonyl content at day 7, 14, 21 and 28.

| | Carbonyl content (nmol carbonyl/mg protein) | | | |
|---|---|---|---|---|
| | Day 7 | Day 14 | Day 21 | Day 28 |
| BSA | 3.02 ± 0.10 | 5.21 ± 0.03 | 8.39 ± 0.01 | 10.04 ± 0.10 |
| BSA + Fructose | 16.28 ± 0.88 | 21.13 ± 1.05 | 23.47 ± 1.27 | 26.28 ± 3.22 |
| BSA + Fructose + KY 0.25 mg/ml | 9.87 ± 0.22# | 13.31 ± 0.76 | 16.58 ± 0.55 | 28.48 ± 0.31 |
| BSA + Fructose + KY 0.50 mg/ml | 9.37 ± 1.49# | 11.45 ± 0.47# | 14.04 ± 0.46 | 27.30 ± 0.45 |
| BSA + Fructose + KY 0.75 mg/ml | 8.31 ± 0.75# | 9.47 ± 0.76 | 13.50 ± 0.72 | 24.24 ± 0.57 |
| BSA + Fructose + KY 1.00 mg/ml | 7.70 ± 0.59# | 8.30 ± 1.40#* | 12.81 ± 0.60# | 23.86 ± 0.60 |
| BSA + Fructose + KY 1.50 mg/ml | 6.77 ± 0.46# | 7.88 ± 0.98#* | 11.86 ± 0.49# | 21.02 ± 0.69 |
| BSA + Fructose + KY 2.00 mg/ml | 5.63 ± 0.45#* | 7.51 ± 1.24#* | 10.38 ± 0.25# | 18.80 ± 0.36# |
| BSA + Fructose + AG 0.50 mg/ml | 8.25 ± 1.11# | 16.44 ± 0.71# | 15.11 ± 0.56# | 23.34 ± 3.48 |
| BSA + Fructose + AG 1.00 mg/ml | 7.86 ± 0.88# | 14.48 ± 2.29# | 16.89 ± 2.46# | 20.69 ± 2.24# |

Data are expressed as mean ± SEM, n =3. #$P < 0.05$ compared to BSA+Fructose and *$P < 0.05$ compared to aminoguanidine 1.00 mg/ml.

**Figure 25 –** The effect of the pomelo cultivars (1.00 mg/ml) on the formations of protein carbonyl content at day 7, 14, 21 and 28 compared to aminoguanidine 1.00 mg/ml




Data are expressed as mean ± SEM, n =3. $^{\#}P < 0.05$ compared to BSA+Fructose and $^{*}P < 0.05$ compared to aminoguanidine 1.00 mg/ml.

### 2.3.2. Formation of thiol groups

The thiol group content in BSA was determined at day 7, 14, 21 and 28. A decrease of thiol groups was observed in BSA + fructose (negative control) (Table 27– 32). The increase in the level of thiol groups by the pomelo cultivar extracts was seen in a concentration dependent manner and the amounts decrease over time (from day 7 to day 28).

On the whole, the pomleo cultivars extract were able to increase the formation of thiol groups by 15% when compared to the BSA+fructose however concentrations that showed significance were 0.75, 1.00, 1.50 and 2.00mg/ml *(P<0.05)* (Table 27 – 32). The highest amount of increase in thiol groups was exerted by the pomelo extract 2.00mg/ml. At day 7, the pomelo cultivars Kao Tanggwa, Tar Koi, Thong Dee, Kao Yai, Kao Nam Pueng, Tubtim Siam at 1.00 mg/ml were able to increase the amount of thiol groups by, 18.94±0.01, 18.01±0.02, 17.94±0.08, 14.40±0.02, 14.40±0.01 and 2.28±0.05% when compared to the BSA + fructose, respectively. Moreover, when compared to aminoguanidine (1.00 mg/ml), the Kao Tanggwa, Tar Koi, Thong Dee, Kao Yai and Kao Nam Pueng at 1.00 mg/ml were able to show a higher ability in increasing the formations of thiol groups by 13.38±0.01, 5.75±0.05, 5.38±0.08, 1.60±0.03 and 0.22±0.02%, respectively. Conversely, the Tubtim Siam at 1.00 mg/ml showed a 10.98±0.08 lesser increasing ability in the thiol groups when compared to the aminoguanidine (1.00mg/ml).

At day 14 which is the day that the pomelo cultivars Kao Tanggwa, Tar Koi, Kao Nam Pueng, Thong Dee, Kao Yai and Tubtim Siam were able to increase the level of formed thiol groups the most with 31.18±0.01, 25.58±0.03, 22.65±0.05, 22.32±0.06, 15.42±0.03 and 15.02±0.03% when compared to the BSA + fructose, respectively. Additionally, the Kao Tanggwa, Tar Koi, Kao Nam Pueng and Thong Dee at 1.00 mg/ml were the pomelo cultivars that were able to show a 16.35±0.05, 8.33±0.05, 5.83±0.05 and 5.58±0.05% higher amount of increasing the formations of thiol groups than aminoguanidine (1.00 mg/ml), respectively. Whereas, the Tubtim

Siam and Kao Yai at 1.00 mg/ml showed a lower ability in increasing the formation of thiol groups than aminoguanidine (1.00 mg/ml) by 3.18±0.07 and 2.73±0.04%, respectively.

At day 21, the pomelo cultivars extract were able to increase the level of thiol groups in the similar pattern, the Kao Tanggwa, Tar Koi, Kao Nam Pueng, Thong Dee, Kao Yai and Tubtim Siam exerted a 29.22±0.07, 22.83±0.03, 21.11±0.09, 21.11±0.01, 2.35±0.06 and 11.25±0.04% when compared to the BSA+fructose, respectively. Furthermore, the Kao Tanggwa was the only pomleo cultivar at 1.00 mg/ml that was able to show a higher ability of increasing the formations of thiol groups than aminoguanidine (1.00 mg/ml) by 1.50±0.01%. In contrast, the Tubtim Siam, Kao Yai, Thong Dee, Kao Nam Pueng and Tar Koi showed a 18.77±0.01, 17.77±0.04, 8.63±0.02, 8.63±0.05 and 6.60±0.03% lesser than the amount exerted by aminoguanidine 1.00 mg/ml, respectively.

At day 28 only the Kao Tanggwa, Kao Nam Pueng, Thong Dee and Tubtim Siam at 1.00 mg/ml were able to increase the level of formed thiol groups by 21.34±0.03, 18.35±0.04, 15.13±0.01 and 0.77±0.01 % when compared to the BSA + fructose. In addition, when compared to aminoguanidine (1.00 mg/ml), the only pomelo cultivar at the equivalent concentration that was able to exert a higher increasing ability in the formation of thiol groups was the Kao Tanggwa and Kao Nam Pueng cultivar which exerted a 6.10±0.02 and 2.53±0.01% higher than the aminoguanidine, respectively. The Kao Yai, Tar Koi, Tubtim Siam and Thong Dee at 1.00 mg/ml, however, showed a 24.68±0.7 23.38±0.05, 15.58±0.02 and 1.30±0.01% lesser amount than aminoguanidine (1.00 mg/ml), respectively (Figure 26).

**Table 28 –** The effect of the pomelo cultivar (Tar Koi) on the formation of thiol groups at day 7, 14, 21 and 28.

| | Thiol group (nmol/mg protein) | | | |
|---|---|---|---|---|
| | Day 7 | Day 14 | Day 21 | Day 28 |
| BSA | 4.37 ± 0.08 | 3.80 ± 0.01 | 3.01 ± 0.12 | 2.72 ± 0.09 |
| BSA + Fructose | 2.14 ± 0.14 | 1.81 ± 0.14 | 1.42 ± 0.22 | 1.29 ± 0.17 |
| BSA + Fructose + TK 0.25 mg/ml | 2.26 ± 0.12 | 2.16 ± 0.19 | 1.20 ± 0.24 | 0.98 ± 0.02 |
| BSA + Fructose + TK 0.50 mg/ml | 2.40 ± 0.41 | 2.21 ± 0.37* | 1.24 ± 0.25 | 1.04 ± 0.03 |
| BSA + Fructose + TK 0.75 mg/ml | 2.57 ± 0.23# | 2.35 ± 0.43# | 1.33 ± 0.46* | 1.08 ± 0.05 |
| BSA + Fructose + TK 1.50 mg/ml | 2.72 ± 0.23# | 2.39 ± 0.14# | 1.52 ± 0.54# | 1.25 ± 0.08# |
| BSA + Fructose + TK 1.00 mg/ml | 2.61 ± 0.41# | 2.43 ± 0.11# | 1.84 ± 0.72# | 1.18 ± 0.21# |
| BSA + Fructose + TK 2.00 mg/ml | 2.80 ± 0.76# | 2.49 ± 0.21# | 1.83 ±0.02# | 1.39 ± 0.07# |
| BSA + Fructose + AG 0.50 mg/ml | 2.38 ± 0.12 | 1.66 ± 0.12 | 1.32 ± 0.61 | 1.06 ± 0.60 |
| BSA + Fructose + AG 1.00 mg/ml | 2.46 ± 0.08# | 2.20 ± 0.08# | 1.97 ± 0.58# | 1.54 ± 0.48# |

Data are expressed as mean ± SEM, n =3. #$P < 0.05$ compared to BSA+Fructose and *$P < 0.05$ compared to aminoguanidine 1.00 mg/ml.

**Table 29** – The effect of the pomelo cultivar (Kao Nam Pueng) on the formation of thiol groups at day 7, 14, 21 and 28.

| | Thiol group (nmol/mg protein) | | | |
|---|---|---|---|---|
| | Day 7 | Day 14 | Day 21 | Day 28 |
| BSA | 4.37 ± 0.08 | 3.80 ± 0.01 | 3.01 ± 0.12 | 2.72 ± 0.09 |
| BSA + Fructose | 2.14 ± 0.14 | 1.81 ± 0.14 | 1.42 ± 0.22 | 1.29 ± 0.17 |
| BSA + Fructose + KNP 0.25 mg/ml | 1.93 ± 0.73 | 1.72 ± 1.02 | 1.26 ± 0.32 | 1.13 ± 0.71 |
| BSA + Fructose + KNP 0.50 mg/ml | 2.09 ± 0.41 | 2.08 ± 0.25 | 1.32 ± 0.54 | 1.22 ± 0.41 |
| BSA + Fructose + KNP 0.75 mg/ml | 2.32 ± 0.70 | 2.22 ± 0.91$^{\#}$ | 1.42 ± 0.12 | 1.32 ± 0.21 |
| BSA + Fructose + KNP 1.00 mg/ml | 2.50 ± 0.40$^{\#}$ | 2.34 ± 0.30$^{\#}$ | 1.80 ± 0.73$^{\#}$ | 1.58 ± 0.14 |
| BSA + Fructose + KNP 1.50 mg/ml | 2.76 ± 0.08$^{\#}$ | 2.45 ± 0.07$^{\#}$ | 1.99 ± 0.43$^{\#}$ | 1.72 ± 0.58$^{\#}$ |
| BSA + Fructose + KNP 2.00 mg/ml | 2.92 ± 0.40$^{\#}$ | 2.56 ± 0.39$^{\#}$ | 2.15 ± 0.27$^{\#}$ | 1.87 ± 0.36$^{\#}$ |
| BSA + Fructose + AG 0.50 mg/ml | 2.38 ± 0.12 | 1.66 ± 0.12 | 1.32 ± 0.61 | 1.06 ± 0.60 |
| BSA + Fructose + AG 1.00 mg/ml | 2.46 ± 0.08$^{\#}$ | 2.20 ± 0.08$^{\#}$ | 1.97 ± 0.58$^{\#}$ | 1.54 ± 0.48$^{\#}$ |

Data are expressed as mean ± SEM, n =3. $^{\#}P < 0.05$ compared to BSA+Fructose and $^{*}P < 0.05$ compared to aminoguanidine 1.00 mg/ml.

**Table 30** – The effect of the pomelo cultivar (Kao Tanggwa) on the formation of thiol groups at day 7, 14, 21 and 28.

| | Thiol group (nmol/mg protein) | | | |
|---|---|---|---|---|
| | Day 7 | Day 14 | Day 21 | Day 28 |
| BSA | 4.37 ± 0.08 | 3.80 ± 0.01 | 3.01 ± 0.12 | 2.72 ± 0.09 |
| BSA + Fructose | 2.14 ± 0.14 | 1.81 ± 0.14 | 1.42 ± 0.22 | 1.29 ± 0.17 |
| BSA + Fructose + KTG 0.25 mg/ml | 2.04 ± 0.22 | 1.85 ± 0.75 | 1.62 ± 0.39 | 1.08 ± 0.09# |
| BSA + Fructose + KTG 0.50 mg/ml | 2.23 ± 0.29 | 1.96 ± 0.26 | 1.75 ± 0.44 | 1.13 ± 0.05# |
| BSA + Fructose + KTG 0.75 mg/ml | 2.36 ± 0.25 | 2.20 ±0.79# | 1.86 ± 0.08 | 1.30 ± 0.11# |
| BSA + Fructose + KTG 1.00 mg/ml | 2.84 ± 0.11# | 2.63 ± 0.18# | 2.00 ± 0.15# | 1.64 ± 0.64# |
| BSA + Fructose + KTG 1.50 mg/ml | 2.94 ± 0.10# | 2.85 ± 0.28# | 2.10 ± 0.18# | 1.92 ± 0.28# |
| BSA + Fructose + KTG 2.00 mg/ml | 3.06 ± 0.25# | 2.94 ± 0.12# | 2.36 ± 0.10# | 2.20 ± 0.17#* |
| BSA + Fructose + AG 0.50 mg/ml | 2.38 ± 0.12 | 1.66 ± 0.12 | 1.32 ± 0.61 | 1.06 ± 0.60 |
| BSA + Fructose + AG 1.00 mg/ml | 2.46 ± 0.08# | 2.20 ± 0.08# | 1.97 ± 0.58# | 1.54 ± 0.48# |

Data are expressed as mean ± SEM, n =3. #$P < 0.05$ compared to BSA+Fructose and *$P < 0.05$ compared to aminoguanidine 1.00 mg/ml.

**Table 31**– The effect of the pomelo cultivar (Thong Dee) on the formation of thiol groups at day 7, 14, 21 and 28.

| | Thiol group (nmol/mg protein) | | | |
|---|---|---|---|---|
| | Day 7 | Day 14 | Day 21 | Day 28 |
| BSA | 4.37 ± 0.08 | 3.80 ± 0.01 | 3.01 ± 0.12 | 2.72 ± 0.09 |
| BSA + Fructose | 2.14 ± 0.14 | 1.81 ± 0.14 | 1.42 ± 0.22 | 1.29 ± 0.17 |
| BSA + Fructose + TD 0.25 mg/ml | 2.46 ± 0.16 | 2.05 ± 0.06 | 1.72 ± 0.14 | 1.31 ± 0.21 |
| BSA + Fructose + TD 0.50 mg/ml | 2.55 ± 0.11$^{\#}$ | 2.23 ± 0.17$^{\#}$ | 1.96 ± 0.47$^{\#}$ | 1.57 ± 0.21$^{\#}$ |
| BSA + Fructose + TD 0.75 mg/ml | 2.70 ± 0.17$^{\#}$ | 2.36 ± 0.16$^{\#}$ | 2.03 ± 0.05$^{\#}$ | 1.70 ± 0.20$^{\#}$ |
| BSA + Fructose + TD 1.00 mg/ml | 2.60 ± 0.42$^{\#}$ | 2.33 ± 0.22$^{\#}$ | 1.80 ± 0.14 | 1.52 ± 0.20 |
| BSA + Fructose + TD 1.50 mg/ml | 2.93 ± 0.14$^{\#}$ | 2.65 ± 0.51$^{\#}$ | 2.38 ± 0.10$^{\#}$ | 2.06 ± 0.20$^{\#}$ |
| BSA + Fructose + TD 2.00 mg/ml | 3.06 ± 0.08$^{\#}$ | 2.89 ± 0.17$^{\#}$ | 2.46 ± 0.15$^{\#*}$ | 2.23 ± 0.11$^{\#*}$ |
| BSA + Fructose + AG 0.50 mg/ml | 2.38 ± 0.12 | 1.66 ± 0.12 | 1.32 ± 0.61 | 1.06 ± 0.60 |
| BSA + Fructose + AG 1.00 mg/ml | 2.46 ± 0.08$^{\#}$ | 2.20 ± 0.08$^{\#}$ | 1.97 ± 0.58$^{\#}$ | 1.54 ± 0.48$^{\#}$ |

Data are expressed as mean ± SEM, n =3. $^{\#}P < 0.05$ compared to BSA+Fructose and $^{*}P < 0.05$ compared to aminoguanidine 1.00 mg/ml.

**Table 32** – The effect of the pomelo cultivar (Tubtim Siam) on the formation of thiol groups at day 7, 14, 21 and 28.

| | Thiol group (nmol/mg protein) | | | |
|---|---|---|---|---|
| | Day 7 | Day 14 | Day 21 | Day 28 |
| BSA | 4.37 ± 0.08 | 3.80 ± 0.01 | 3.01 ± 0.12 | 2.72 ± 0.09 |
| BSA + Fructose | 2.14 ± 0.14 | 1.81 ± 0.14 | 1.42 ± 0.22 | 1.29 ± 0.17 |
| BSA + Fructose + TS 0.25 mg/ml | 2.01 ± 0.06 | 1.45 ± 0.23 | 1.37 ± 0.19 | 1.06 ± 0.13 |
| BSA + Fructose + TS 0.50 mg/ml | 2.03 ± 0.18 | 1.67 ± 0.21 | 1.49 ± 0.23 | 1.24 ± 0.08 |
| BSA + Fructose + TS 0.75 mg/ml | 2.11 ± 0.08 | 1.79 ± 0.21 | 1.60 ± 0.09 | 1.30 ± 0.02 |
| BSA + Fructose + TS 1.00 mg/ml | 2.19 ± 0.32 | 2.03 ± 0.32 | 1.70 ± 0.08$^{\#}$ | 1.60 ± 0.50 |
| BSA + Fructose + TS 1.50 mg/ml | 2.47 ± 0.27$^{\#}$ | 2.22 ± 0.53$^{\#}$ | 1.83 ± 0.27$^{\#}$ | 1.66 ± 0.23$^{\#}$ |
| BSA + Fructose + TS 2.00 mg/ml | 2.78 ± 0.26$^{\#}$ | 2.52 ± 0.44$^{\#}$ | 2.2 ± 0.34$^{\#}$ | 1.80 ± 0.02$^{\#}$ |
| BSA + Fructose + AG 0.50 mg/ml | 2.38 ± 0.12 | 1.66 ± 0.12 | 1.32 ± 0.61 | 1.06 ± 0.60 |
| BSA + Fructose + AG 1.00 mg/ml | 2.46 ± 0.08$^{\#}$ | 2.20 ± 0.08$^{\#}$ | 1.97 ± 0.58$^{\#}$ | 1.54 ± 0.48$^{\#}$ |

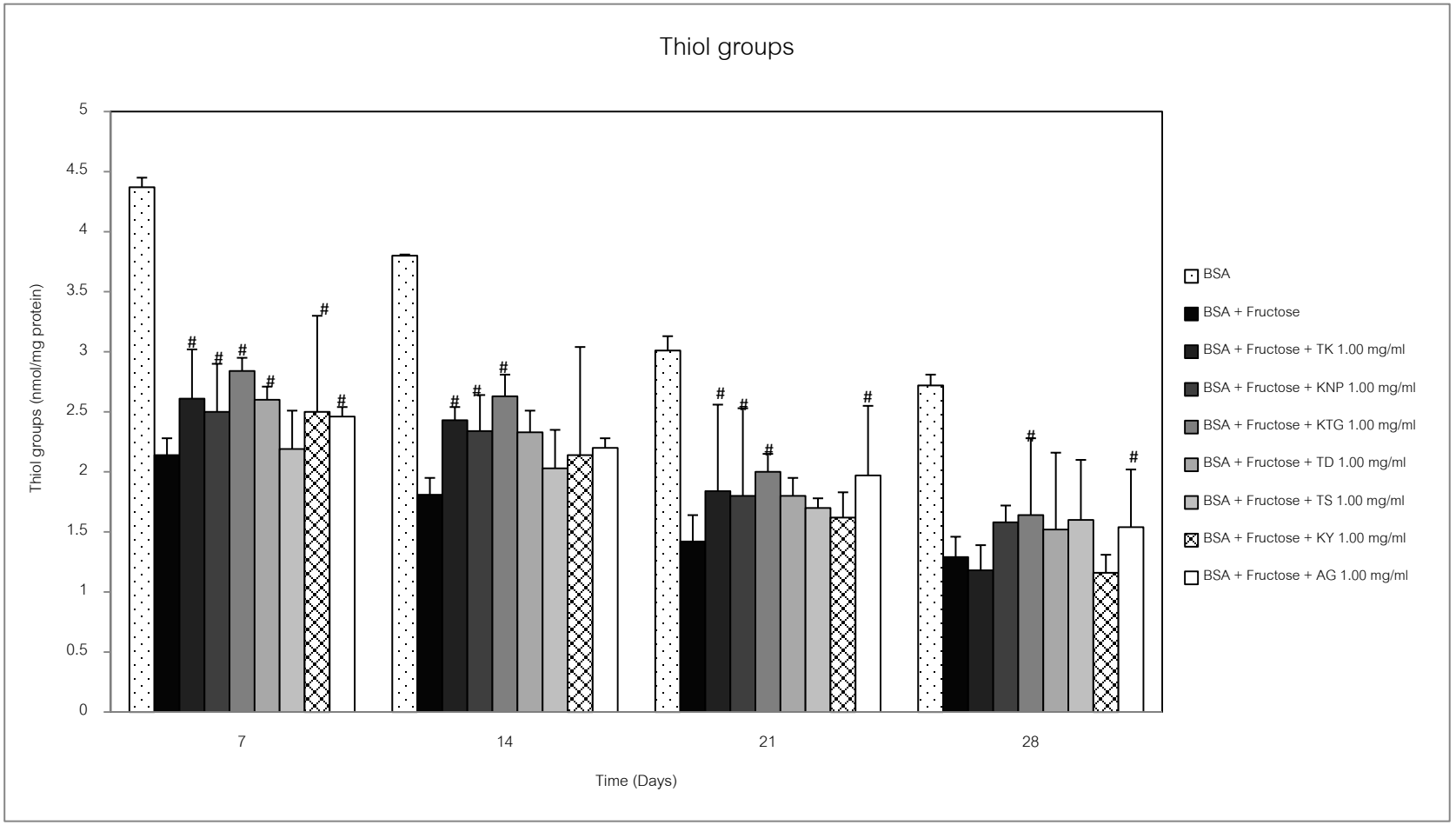
Data are expressed as mean ± SEM, n =3. $^{\#}P < 0.05$ compared to BSA+Fructose and $^{*}P < 0.05$ compared to aminoguanidine 1.00 mg/ml.

**Table 33** – The effect of the pomelo cultivar (Kao Yai) on the formation of thiol groups at day 7, 14, 21 and 28.

| | Thiol group (nmol/mg protein) | | | |
|---|---|---|---|---|
| | Day 7 | Day 14 | Day 21 | Day 28 |
| BSA | 4.37 ± 0.08 | 3.80 ± 0.01 | 3.01 ± 0.12 | 2.72 ± 0.09 |
| BSA + Fructose | 2.14 ± 0.14 | 1.81 ± 0.14 | 1.42 ± 0.22 | 1.29 ± 0.17 |
| BSA + Fructose + KY 0.25 mg/ml | 2.15 ± 0.20 | 1.55 ± 0.20 | 1.38 ± 0.25 | 0.76 ± 0.36 |
| BSA + Fructose + KY 0.50 mg/ml | 2.29 ± 0.83 | 1.88 ± 0.83 | 1.45 ± 0.38 | 0.81 ± 0.13 |
| BSA + Fructose + KY 0.75 mg/ml | 2.39 ± 0.49 | 2.04 ± 0.49 | 1.58 ± 0.41 | 0.99 ± 0.28 |
| BSA + Fructose + KY 1.00 mg/ml | 2.50 ± 1.08$^{\#}$ | 2.14 ± 1.08$^{\#}$ | 1.62 ± 0.21 | 1.16 ± 0.15 |
| BSA + Fructose + KY 1.50 mg/ml | 2.71 ± 0.74$^{\#}$ | 2.23 ± 0.74$^{\#}$ | 1.80 ± 0.56$^{\#}$ | 1.38 ± 0.34$^{\#}$ |
| BSA + Fructose + KY 2.00 mg/ml | 2.99 ± 0.49$^{\#*}$ | 2.42 ± 0.49$^{\#}$ | 1.92 ± 0.74$^{\#}$ | 1.55 ± 0.28$^{\#}$ |
| BSA + Fructose + AG 0.50 mg/ml | 2.38 ± 0.12 | 1.66 ± 0.12 | 1.32 ± 0.61 | 1.06 ± 0.60 |
| BSA + Fructose + AG 1.00 mg/ml | 2.46 ± 0.08$^{\#}$ | 2.20 ± 0.08$^{\#}$ | 1.97 ± 0.58$^{\#}$ | 1.54 ± 0.48$^{\#}$ |

Data are expressed as mean ± SEM, n =3. $^{\#}P < 0.05$ compared to BSA+Fructose and $^{*}P < 0.05$ compared to aminoguanidine 1.00 mg/ml.

**Figure 26** - The effect of the pomelo cultivars (1.00 mg/ml) on the formations of thiol groups at day 7, 14, 21 and 28 compared to aminoguanidine 1.00 mg/ml




Data are expressed as mean ± SEM, n =3. $^{\#}P < 0.05$ compared to BSA+Fructose and $^{*}P < 0.05$ compared to aminoguanidine 1.00 mg/ml.

### 2.4. Measurement of β-amyloid structures

As shown table 32 -37 and figure 27 - 32, the formation of β-amyloid structures formed as a result of protein oxidation derived from glycation was determined at day 7, 14, 21 and 28. An increase in β-amyloid structures was observed in the incubation of BSA + fructose (negative control). Inhibition of β-amyloid structures by the pomelo extract was in a concentration dependent manner.

All six pomelo cultivars at all concentrations (0.25, 0.50, 0.75, 1.00, 1.50 and 2.00mg/ml) were able to show a significance decrease in the formation β-amyloid structures when compared to the BSA+fructose (negative control). At day 7, the pomelo cultivars extract Thong Dee, Kao Tanggwa, Kao Nam Pueng, Kao Yai, Tar Koi and Tubtim Siam at 1.00 mg/ml were able to exert a 83.27±0.02, 81.40±0.05, 49.83±0.09, 47.56±0.08, 44.30±0.03 and 41.68±0.01% of decrease in the formations of β-amyloid structures when compared to the BSA + fructose, respectively.

At day 14 in the same pattern, the pomelo cultivars Thong Dee, Kao Tanggwa, Kao Yai, Kao Nam Pueng, Tar Koi and Tubtim Siam at 1.00 mg/ml exerted a 73.56±0.01, 70.56±0.02, 64.89±0.04, 58.40±0.07, 54.44±0.09 and 41.10±0.04% of a decrease in the formation of β-amyloid structures when compared to the BSA + fructose, respectively.

At day 21, the pomelo cultivars extract Thong Dee, Kao Tanggwa, Kao Yai, Kao Nam Pueng, Tar Koi and Tubtim Siam at 1.00 mg/ml showed a 72.13±0.04, 68.41±0.05, 63.90±0.07, 61.36±0.01, 56.19±0.01 and 42.96±0.03 %, decrease in the formation of β-amyloid structures when compared to the BSA + fructose, respectively.

At day 28, in a similar manner when compared to the BSA + fructose, the pomelo cultivars extract at 1.00 mg/ml Thong Dee, Kao Tanggwa, Kao Nampueng, Kao Yai, Tar Koi and Tubtim Siam exerted a 73.39±0.01, 67.40±0.01, 64.80±0.04, 64.53±0.02, 55.66±0.01 and 39.58±0.01 % decrease in the formations of β-amyloid structures, respectively.

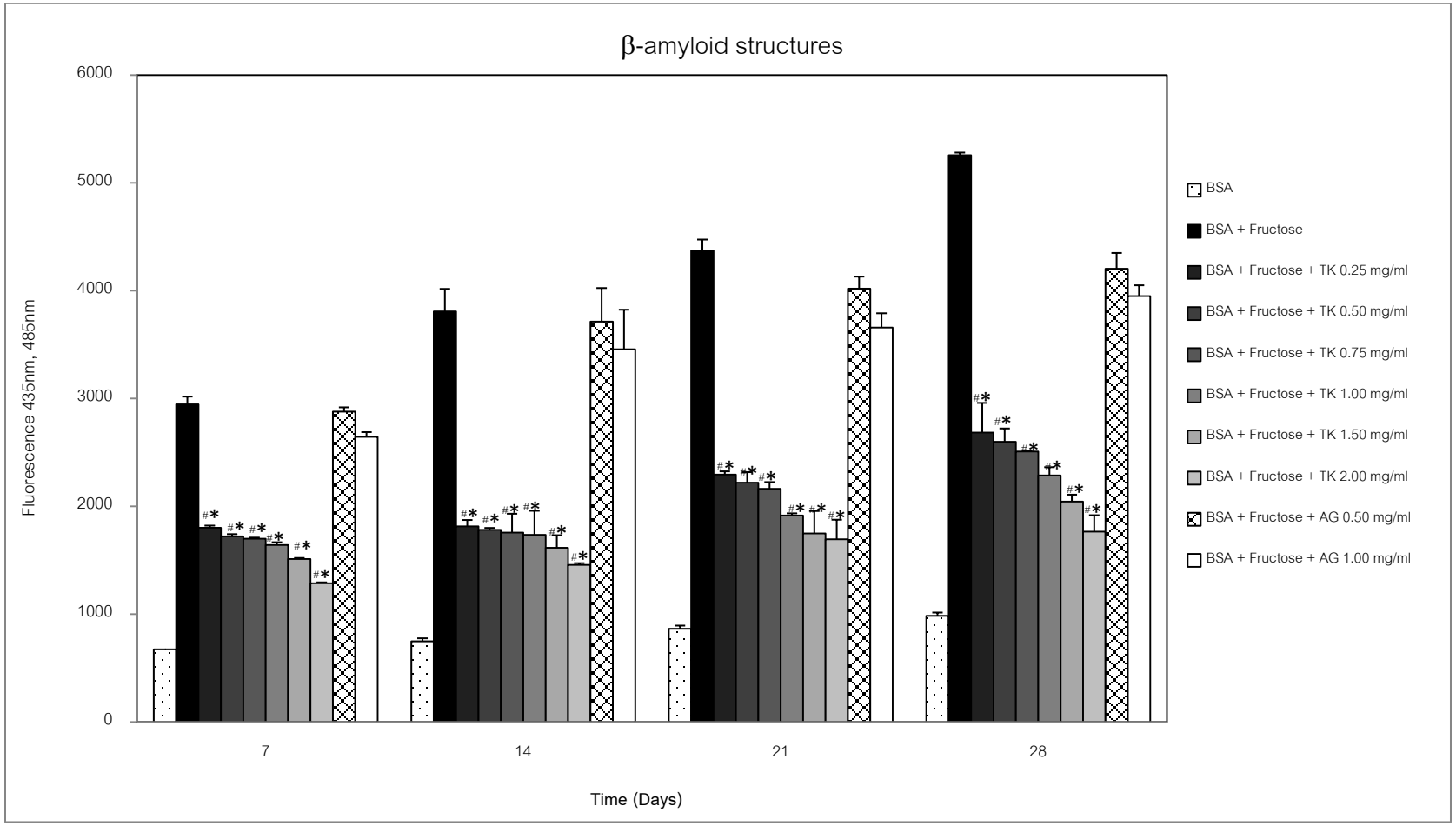
The aminoguanidine (1.00 mg/ml) had also showed decrease formations in the β-amyloid structures by only 10.29 – 23.41% from day 7 to day 28 and was not significant when compared to the BSA+Fructose. Notably the all of the six pomelo cultivars extract at the concentration of 1.00 mg/ml were able to exert a significantly higher decreasing power than that of aminoguanidine (1.00 mg/ml), in which the Thong Dee and Kao Tanggwa, two of the pomelo cultivars that exerted the highest decreasing ability in the formations of β-amyloid structures, exhibited an approximate 65.26 – 81.35 and 57.43 - 79.27% higher ability of decrease than exerted by aminoguanidine ($P<0.01$). The other pomelo cultivars also at 1.00 mg/ml were able to exert a 21.12 – 61.31% significantly higher amount than that of aminoguanidine (1.00 mg/ml) ($P<0.01$) (Figure 33).

**Table 34 –** The effect of pomelo cultivar (Tar Koi) on the formation of β-amyloid structures at day 7, 14, 21 and 28.

| | β-amyloid structures (nm) | | | |
|---|---|---|---|---|
| | Day 7 | Day 14 | Day 21 | Day 28 |
| BSA | 673.12 ± 28.11 | 748.21 ± 30.12 | 864.01 ± 31.21 | 984.12 ± 39.56 |
| BSA + Fructose | 2,946.21 ± 71.63 | 3,808.10 ± 208.85 | 4,371.10 ± 103.24 | 5,156.23 ± 24.92 |
| BSA + Fructose + TK 0.25 mg/ml | 1,800.33 ± 20.90$^{\#*}$ | 1,894.68 ± 59.41$^{\#*}$ | 2,294.23 ± 30.66$^{\#*}$ | 2,683.35 ± 275.39$^{\#*}$ |
| BSA + Fructose + TK 0.50 mg/ml | 1,721.37 ± 20.67$^{\#*}$ | 1,781.81 ± 18.50$^{\#*}$ | 2,219.67 ± 97.59$^{\#*}$ | 2,599.39 ± 123.14$^{\#*}$ |
| BSA + Fructose + TK 0.75 mg/ml | 1,699.66 ± 10.82$^{\#*}$ | 1,756.12 ± 113.88$^{\#*}$ | 2,163.37 ± 62.13$^{\#*}$ | 2,5087.21 ± 5.61$^{\#*}$ |
| BSA + Fructose + TK 1.00 mg/ml | 1,641,89 ± 25.83$^{\#*}$ | 1,735.56 ± 120.8$^{\#*}$ | 1,915.33 ± 20.03$^{\#*}$ | 2,286.29 ± 77.51$^{\#*}$ |
| BSA + Fructose + TK 1.50 mg/ml | 1,510.82 ± 10.67$^{\#*}$ | 1,615.44 ± 99.7$^{\#*}$ | 1,747.56 ± 208.73$^{\#*}$ | 2,043.67 ± 64.38$^{\#*}$ |
| BSA + Fructose + TK 2.00 mg/ml | 1,286.13 ± 8.41$^{\#*}$ | 1,456.32 ± 17.16$^{\#*}$ | 1,693.78 ± 182.18$^{\#*}$ | 1,764.67 ± 152.88$^{\#*}$ |
| BSA + Fructose + AG 0.50 mg/ml | 2,878.55 ± 40.38 | 3,713.34 ± 311.67 | 4,019.65 ± 111.32 | 4,203.31 ± 147.87 |
| BSA + Fructose + AG 1.00 mg/ml | 2,643.23 ± 45.26 | 3,456.56 ± 367.52 | 3,658.87 ± 132.55 | 3,949.02 ± 101.10 |

Data are expressed as mean ± SEM, n =3.* $^{\#}P < 0.01$ compared to BSA+Fructose and $^{*}P < 0.01$ compared to aminoguanidine 1.00 mg/ml.

**Figure 27** - The effect of pomelo cultivar (Tar Koi) on the formation of β-amyloid structures at day 7, 14, 21 and 28.



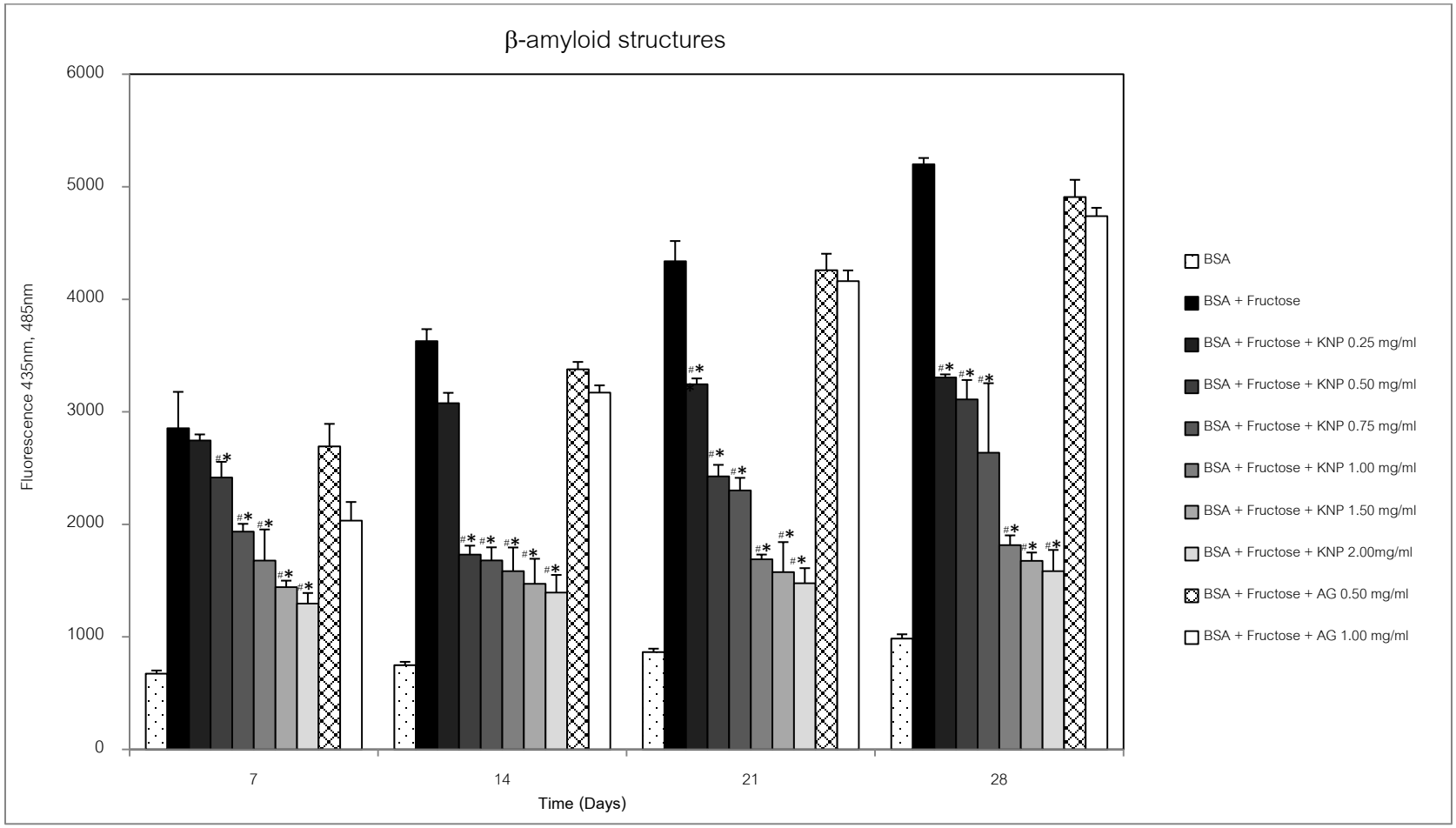

Data are expressed as mean ± SEM, n =3. $^{\#}P < 0.01$ compared to BSA+Fructose and $^{*}P < 0.01$ compared to aminoguanidine 1.00 mg/ml.

**Table 35 –** The effect of pomelo cultivar (Kao Nam Pueng) on the formation of β-amyloid structures at day 7, 14, 21 and 28.

| | β-amyloid structures (nm) | | | |
|---|---|---|---|---|
| | Day 7 | Day 14 | Day 21 | Day 28 |
| BSA | 673.12 ± 28.11 | 748.21 ± 30.12 | 864.01 ± 31.21 | 984.12 ± 39.56 |
| BSA + Fructose | 2,946.21 ± 71.63 | 3,808.10 ± 208.85 | 4,371.10 ± 103.24 | 5,156.23 ± 24.92 |
| BSA + Fructose + KNP 0.25 mg/ml | 1,844.60 ± 91.80$^{\#*}$ | 2,077.21 ± 51.54$^{\#*}$ | 3,245.12 ± 26.59$^{\#*}$ | 3,306.33 ± 52.48$^{\#*}$ |
| BSA + Fructose + KNP 0.50 mg/ml | 1,616.45 ± 70.59$^{\#*}$ | 1,731.20 ± 101.66$^{\#*}$ | 2,427.17 ± 172.83$^{\#*}$ | 3,110.12 ± 236.04$^{\#*}$ |
| BSA + Fructose + KNP 0.75 mg/ml | 1,536.21 ± 115.57$^{\#*}$ | 1,680.19 ± 112.15$^{\#*}$ | 2,301.23 ± 167.12$^{\#*}$ | 2,637.34 ± 218.88$^{\#*}$ |
| BSA + Fructose + KNP 1.00 mg/ml | 1,478.90 ± 210.25$^{\#*}$ | 1,584.23 ± 42.49$^{\#*}$ | 1,689.34 ± 86.91$^{\#*}$ | 1,815.88 ± 66.64$^{\#*}$ |
| BSA + Fructose + KNP 1.50 mg/ml | 1,342.77 ± 221.97$^{\#*}$ | 1,472.31 ± 266.16$^{\#*}$ | 1,576.33 ± 73.39$^{\#*}$ | 1,676.98 ± 184.32$^{\#*}$ |
| BSA + Fructose + KNP 2.00 mg/ml | 1,296.67 ± 156.39$^{\#*}$ | 1,394.20 ± 133.72$^{\#*}$ | 1,477.43 ± 188.37$^{\#*}$ | 1,584.76 ± 113.88$^{\#*}$ |
| BSA + Fructose + AG 0.50 mg/ml | 2,878.55 ± 40.38 | 3,713.34 ± 311.67 | 4,019.65 ± 111.32 | 4,203.31 ± 147.87 |
| BSA + Fructose + AG 1.00 mg/ml | 2,643.23 ± 45.26 | 3,456.56 ± 367.52 | 3,658.87 ± 132.55 | 3,949.02 ± 101.10 |

Data are expressed as mean ± SEM, n =3.* $^{\#}P < 0.01$ compared to BSA+Fructose and $^{*}P < 0.01$ compared to aminoguanidine 1.00 mg/ml.

**Figure 28 –** The effect of pomelo cultivar (Kao Nam Pueng) on the formation of β-amyloid structures at day 7, 14, 21 and 28.



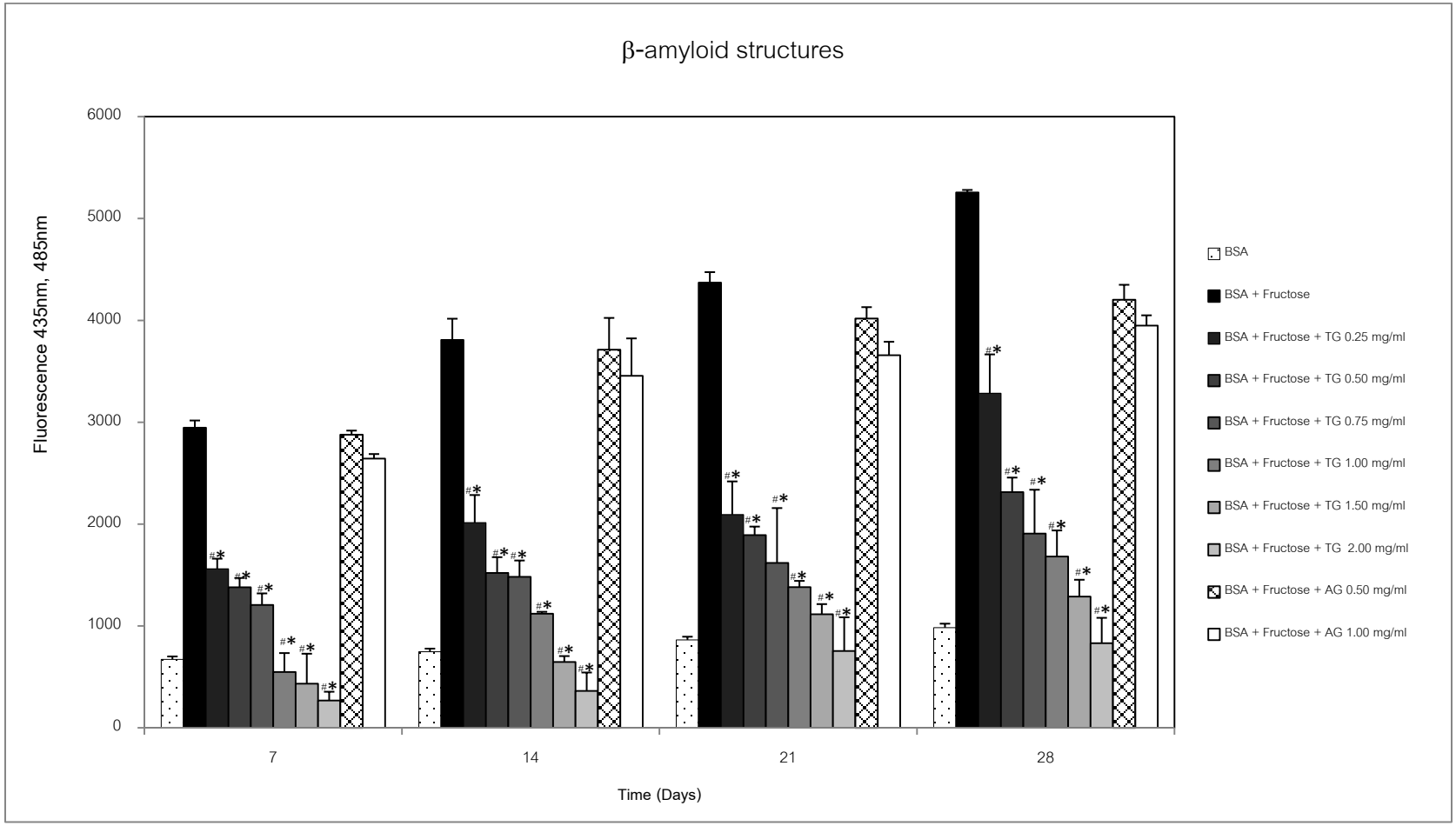

Data are expressed as mean ± SEM, n =3. $^{\#}P < 0.01$ compared to BSA+Fructose and $^{*}P < 0.01$ compared to aminoguanidine 1.00 mg/ml.

**Table 36 –** The effect of pomelo cultivar (Kao Tang Gwa) on the formation of β-amyloid structures at day 7, 14, 21 and 28.

| β-amyloid structures (nm) | | | | |
|---|---|---|---|---|
| | Day 7 | Day 14 | Day 21 | Day 28 |
| BSA | 673.12 ± 28.11 | 748.21 ± 30.12 | 864.01 ± 31.21 | 984.12 ± 39.56 |
| BSA + Fructose | 2,946.21 ± 71.63 | 3,808.10 ± 208.85 | 4,371.10 ± 103.24 | 5,156.23 ± 24.92 |
| BSA + Fructose + KTG 0.25 mg/ml | 1,560.12 ± 100.6$^{\#*}$ | 2,014.23 ± 271.91$^{\#*}$ | 2,293.32 ± 126.51$^{\#*}$ | 3,283.32 ± 54.87$^{\#*}$ |
| BSA + Fructose + KTG 0.50 mg/ml | 1,380.27 ± 91.92$^{\#*}$ | 1,520.38 ± 155.83$^{\#*}$ | 1,891.22 ± 84.72$^{\#*}$ | 2,314.21 ± 383.11$^{\#*}$ |
| BSA + Fructose + KTG 0.75 mg/ml | 1,207.12 ± 113.35$^{\#*}$ | 1,482.23 ± 161.38$^{\#*}$ | 1,619.34 ± 138.78$^{\#*}$ | 1,908.34 ± 143.59$^{\#*}$ |
| BSA + Fructose + KTG 1.00 mg/ml | 548.11 ± 186.36$^{\#*}$ | 1,121.34 ± 18.67$^{\#*}$ | 1,381.33 ± 61.55$^{\#*}$ | 1,681.55 ± 430.19$^{\#*}$ |
| BSA + Fructose + KTG 1.50 mg/ml | 433.34 ± 294.77$^{\#*}$ | 646.76 ± 58.77$^{\#*}$ | 1,115.12 ± 100$^{\#*}$ | 1,289.30 ± 256.75$^{\#*}$ |
| BSA + Fructose + KTG 2.00 mg/ml | 269.23 ± 86.10$^{\#*}$ | 362.89 ± 181.78$^{\#*}$ | 754.45 ± 331.11$^{\#*}$ | 831.23 ± 165.33$^{\#*}$ |
| BSA + Fructose + AG 0.50 mg/ml | 2,878.55 ± 40.38 | 3,713.34 ± 311.67 | 4,019.65 ± 111.32 | 4,203.31 ± 147.87 |
| BSA + Fructose + AG 1.00 mg/ml | 2,643.23 ± 45.26 | 3,456.56 ± 367.52 | 3,658.87 ± 132.55 | 3,949.02 ± 101.10 |

Data are expressed as mean ± SEM, n =3.* $^{\#}P < 0.01$ compared to BSA+Fructose and *$P < 0.01$ compared to aminoguanidine 1.00 mg/ml.

**Figure 29** – The effect of pomelo cultivar (Kao Tanggwa) on the formation of β-amyloid structures at day 7, 14, 21 and 28.



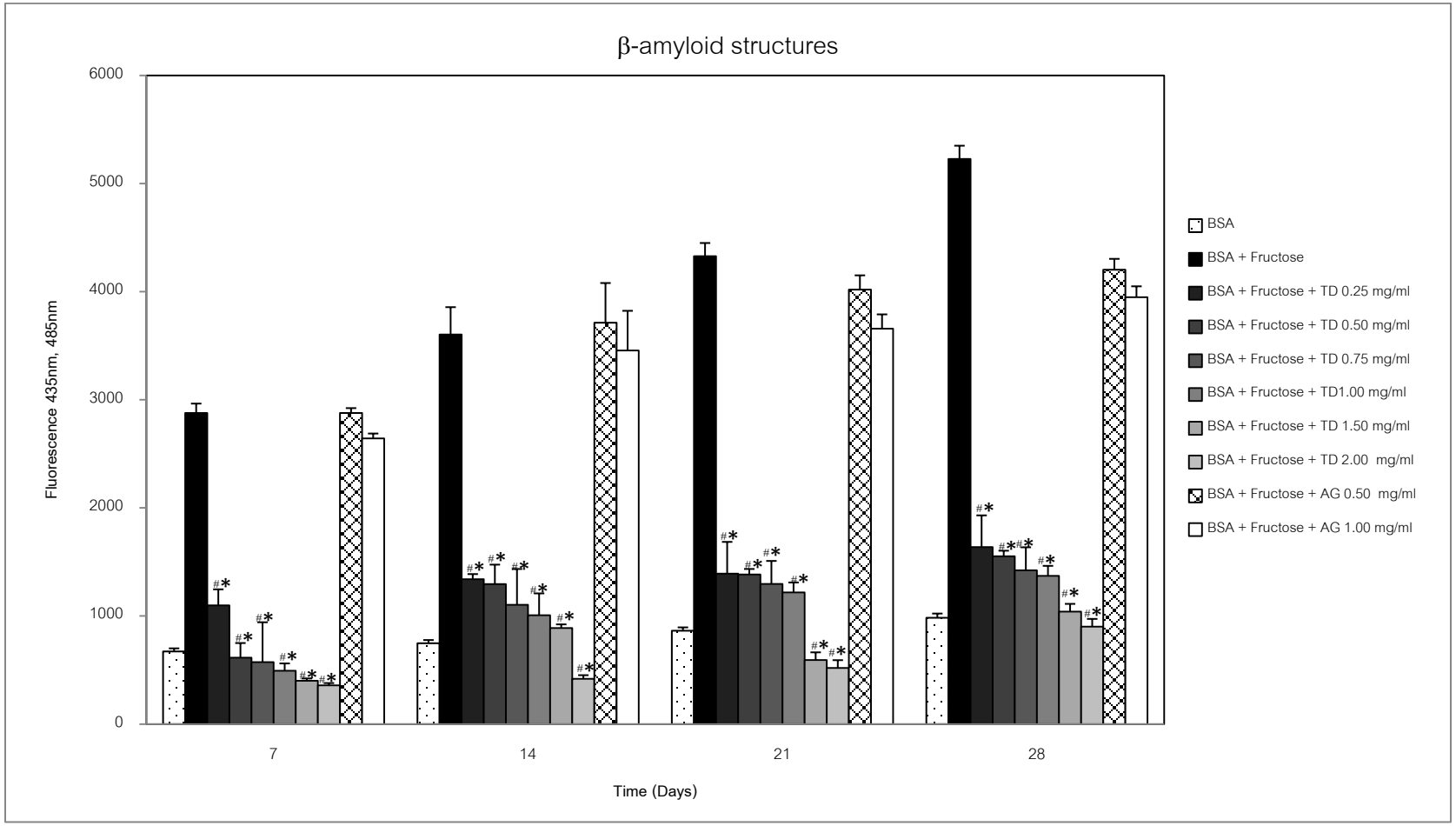

Data are expressed as mean ± SEM, n =3. $^{\#}P < 0.01$ compared to BSA+Fructose and $^{*}P < 0.01$ compared to aminoguanidine 1.00 mg/ml.

**Table 37 –** The effect of pomelo cultivar (Thong Dee) on the formation of β-amyloid structures at day 7, 14, 21 and 28.

| | β-amyloid structures (nm) | | | |
|---|---|---|---|---|
| | Day 7 | Day 14 | Day 21 | Day 28 |
| BSA | 673.12 ± 28.11 | 748.21 ± 30.12 | 864.01 ± 31.21 | 984.12 ± 39.56 |
| BSA + Fructose | 2,946.21 ± 71.63 | 3,808.10 ± 208.85 | 4,371.10 ± 103.24 | 5,156.23 ± 24.92 |
| BSA + Fructose + TD 0.25 mg/ml | 1,099.11 ± 148.43$^{\#*}$ | 1,340.20 ± 48.06$^{\#*}$ | 1,392.21 ± 293.55$^{\#*}$ | 1,638.34 ± 293.55$^{\#*}$ |
| BSA + Fructose + TD 0.50 mg/ml | 615.45 ± 134.54$^{\#*}$ | 1,295.34 ± 180.82$^{\#*}$ | 1,384.22 ± 51.32$^{\#*}$ | 1,553.56 ± 92.45$^{\#*}$ |
| BSA + Fructose + TD 0.75 mg/ml | 573.65 ± 16.99$^{\#*}$ | 1,104.45 ± 110.20$^{\#*}$ | 1,296.45 ± 213.27$^{\#*}$ | 1,423.98 ± 51.32$^{\#*}$ |
| BSA + Fructose + TD 1.00 mg/ml | 493.51 ± 68.20$^{\#*}$ | 1,007.85 ± 331.20$^{\#*}$ | 1,218.40 ± 92.55$^{\#*}$ | 1,372.54 ± 213.27$^{\#*}$ |
| BSA + Fructose + TD 1.50 mg/ml | 401.45 ± 84.87$^{\#*}$ | 889.43 ± 202.47$^{\#*}$ | 592.44 ± 230.96$^{\#*}$ | 1040.43 ± 92.55$^{\#*}$ |
| BSA + Fructose + TD 2.00 mg/ml | 358.23 ± 22.12$^{\#*}$ | 419.40 ± 338.34$^{\#*}$ | 520.67 ± 72.16$^{\#*}$ | 901.32 ± 230.96$^{\#*}$ |
| BSA + Fructose + AG 0.50 mg/ml | 2,878.55 ± 40.38 | 3,713.34 ± 311.67 | 4,019.65 ± 111.32 | 4,203.31 ± 147.87 |
| BSA + Fructose + AG 1.00 mg/ml | 2,643.23 ± 45.26 | 3,456.56 ± 367.52 | 3,658.87 ± 132.55 | 3,949.02 ± 101.10 |

Data are expressed as mean ± SEM, n =3. $^{\#}P < 0.01$ compared to BSA+Fructose and $^{*}P < 0.01$ compared to aminoguanidine 1.00 mg/ml.

**Figure 30** – The effect of pomelo cultivar (Thong Dee) on the formation of β-amyloid structures at day 7, 14, 21 and 28.



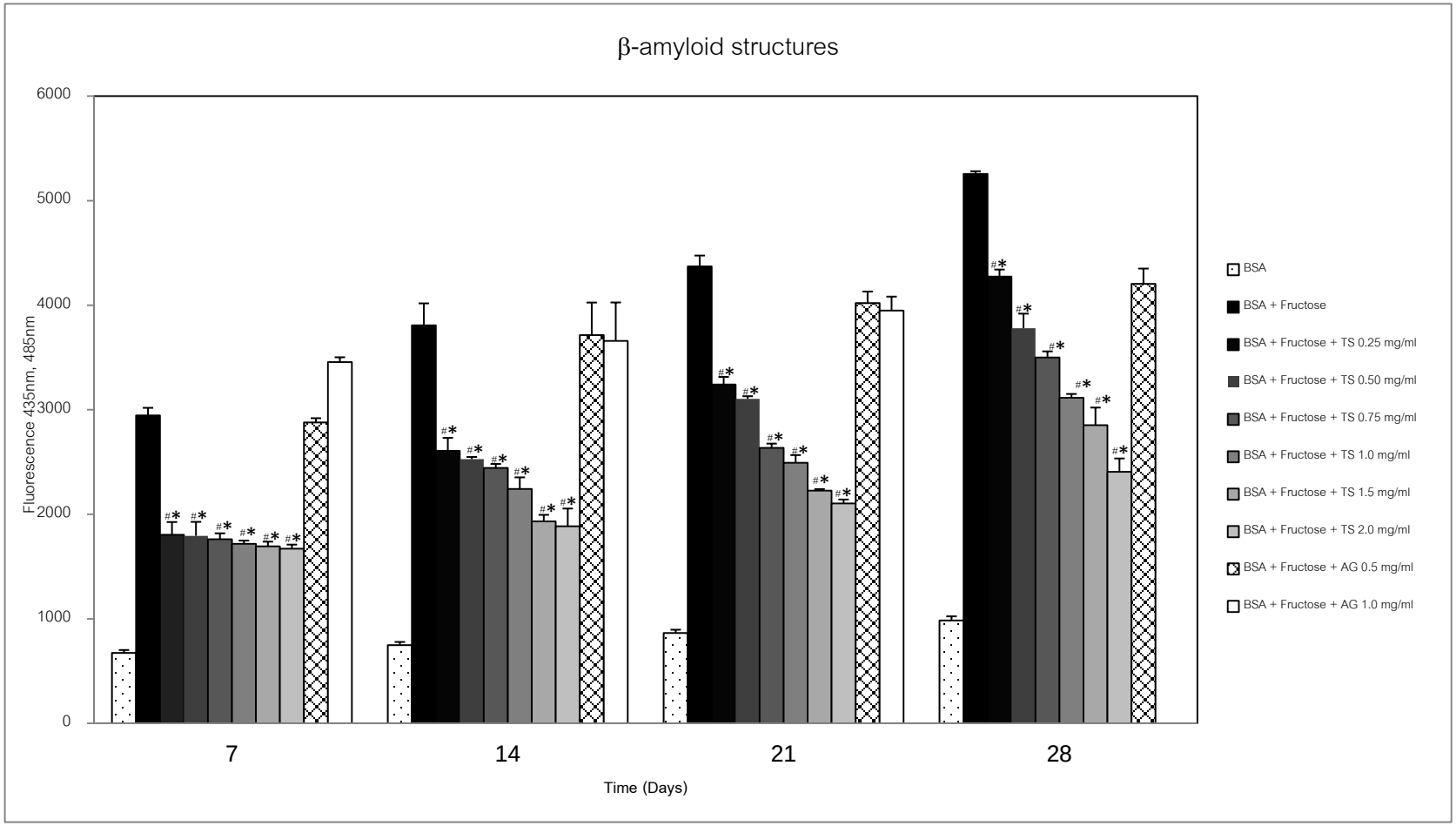

Data are expressed as mean ± SEM, n =3. $^{\#}P < 0.01$ compared to BSA+Fructose and $^{*}P < 0.01$ compared to aminoguanidine 1.00 mg/ml.

**Table 38 –** The effect of pomelo cultivar (Tubtim Siam) on the formation of β-amyloid structures at day 7, 14, 21 and 28.

| β-amyloid structures (nm) | | | | |
|---|---|---|---|---|
| | Day 7 | Day 14 | Day 21 | Day 28 |
| BSA | 673.12 ± 28.11 | 748.21 ± 30.12 | 864.01 ± 31.21 | 984.12 ± 39.56 |
| BSA + Fructose | 2,946.21 ± 71.63 | 3,808.10 ± 208.85 | 4,371.10 ± 103.24 | 5,156.23 ± 24.92 |
| BSA + Fructose + TS 0.25 mg/ml | 1,805.12 ± 120.50$^{\#*}$ | 2,608.21 ± 122.58$^{\#*}$ | 3,241.21 ± 72.34 | 4,273.20 ± 67.27 |
| BSA + Fructose + TS 0.50 mg/ml | 1,793.23 ± 133.41$^{\#*}$ | 2,527.22 ± 20.22$^{\#*}$ | 3,104.34 ± 25.20$^{\#*}$ | 3,780.55 ± 138.84 |
| BSA + Fructose + TS 0.75 mg/ml | 1,762.45 ± 55.43 $^{\#*}$ | 2,443.45 ± 37.55$^{\#*}$ | 2,635.55 ± 40.25$^{\#*}$ | 3,500.45 ± 58.01 |
| BSA + Fructose + TS 1.00 mg/ml | 1,718.32 ± 29.55$^{\#*}$ | 2,243.32 ± 109.88$^{\#*}$ | 2,493.67 ± 72.43$^{\#*}$ | 3,115.21 ± 35.53$^{\#*}$ |
| BSA + Fructose + TS 1.50 mg/ml | 1,693.45 ± 44.44$^{\#*}$ | 1,931.13 ± 62.76$^{\#*}$ | 2,227.45 ± 13.5$^{\#*}$7 | 2,852.29 ± 167.75$^{\#*}$ |
| BSA + Fructose + TS 2.00 mg/ml | 1,671.76 ± 38.63$^{\#*}$ | 1,885.32 ± 169.01$^{\#*}$ | 2,104.55 ± 37$^{\#*}$ | 2,406.43 ± 127.87$^{\#*}$ |
| BSA + Fructose + AG 0.50 mg/ml | 2,878.55 ± 40.38 | 3,713.34 ± 311.67 | 4,019.65 ± 111.32 | 4,203.31 ± 147.87 |
| BSA + Fructose + AG 1.00 mg/ml | 2,643.23 ± 45.26 | 3,456.56 ± 367.52 | 3,658.87 ± 132.55 | 3,949.02 ± 101.10 |

Data are expressed as mean ± SEM, n =3. $^{\#}P < 0.01$ compared to BSA+Fructose and $^{*}P < 0.01$ compared to aminoguanidine 1.00 mg/ml.

**Figure 31** – The effect of pomelo cultivar (Tubtim Siam) on the formation of β-amyloid structures at day 7, 14, 21 and 28.



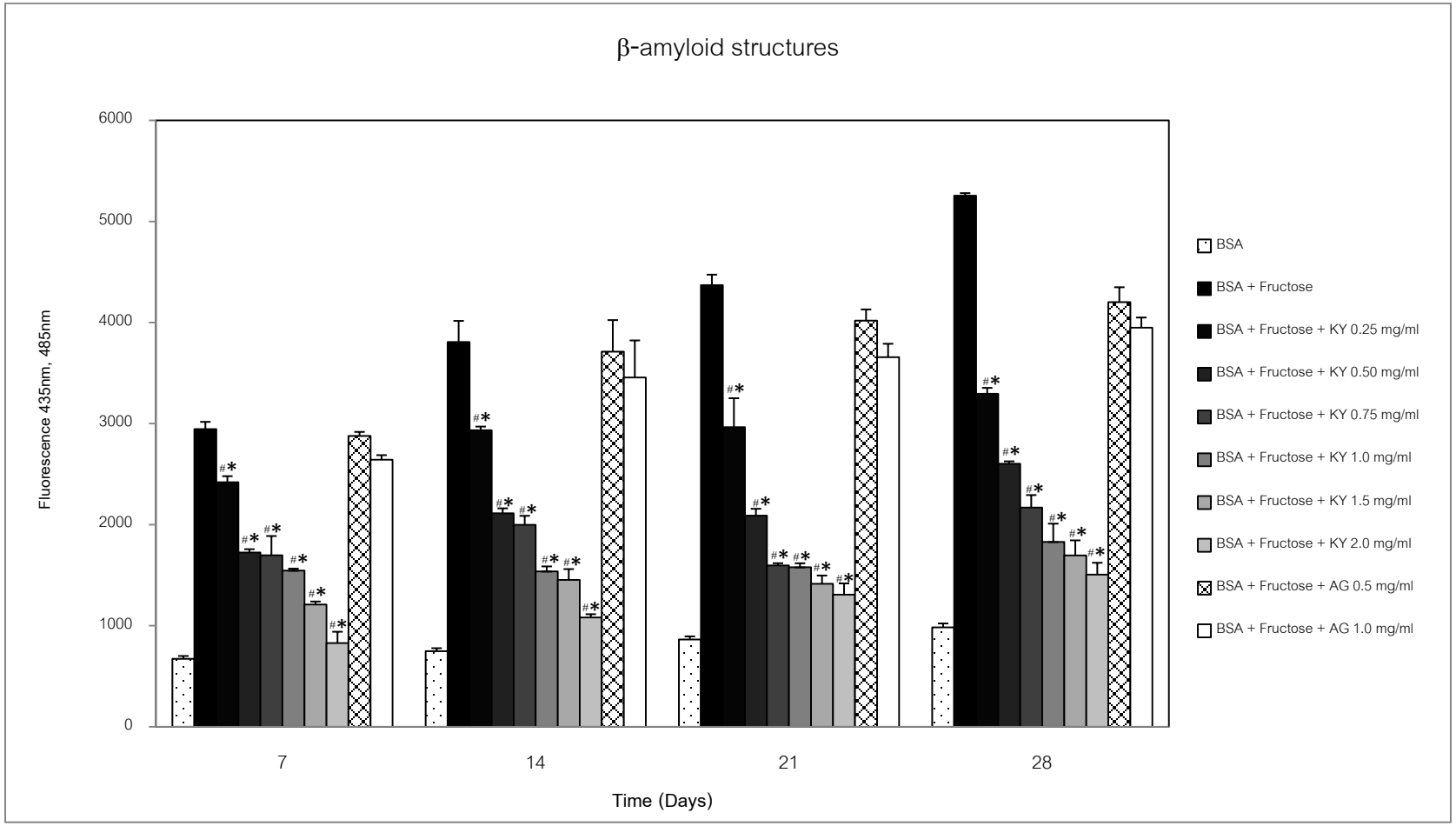

Data are expressed as mean ± SEM, n =3. $^{\#}P < 0.01$ compared to BSA+Fructose and $^{*}P < 0.01$ compared to aminoguanidine 1.00 mg/ml.

**Table 39 –** The effect of pomelo cultivar (Kao Yai) on the formation of β-amyloid structures at day 7, 14, 21 and 28.

| β-amyloid structures (nm) | | | | |
|---|---|---|---|---|
| | Day 7 | Day 14 | Day 21 | Day 28 |
| BSA | 673.12 ± 28.11 | 748.21 ± 30.12 | 864.01 ± 31.21 | 984.12 ± 39.56 |
| BSA + Fructose | 2,946.21 ± 71.63 | 3,808.10 ± 208.85 | 4,371.10 ± 103.24 | 5,156.23 ± 24.92 |
| BSA + Fructose + KY 0.25 mg/ml | 2,020.45 ± 60.41$^{\#}$ | 2,934.34 ± 36.84$^{\#*}$ | 2,965.27 ± 286.70$^{\#*}$ | 3,295.23 ± 59$^{\#*}$ |
| BSA + Fructose + KY 0.50 mg/ml | 1,725.34 ± 32.62$^{\#}$ | 2,113.56 ± 49$^{\#*}$ | 2,090.34 ± 67.58$^{\#*}$ | 2,603.45 ± 200$^{\#*}$ |
| BSA + Fructose + KY 0.75 mg/ml | 1,697.45 ± 190.97$^{\#*}$ | 1,999.89 ± 88.88$^{\#*}$ | 1,597.55 ± 21.96$^{\#*}$ | 2,169.59 ± 124.30$^{\#*}$ |
| BSA + Fructose + KY 1.00 mg/ml | 1,545.55 ± 20.48$^{\#*}$ | 1,337.87 ± 50.45$^{\#*}$ | 1,578.32 ± 40.34$^{\#*}$ | 1,829.78 ± 181.47$^{\#*}$ |
| BSA + Fructose + KY 1.50 mg/ml | 1,210.32 ± 29.79$^{\#*}$ | 1,454.77 ± 106.78$^{\#*}$ | 1,415.43 ± 82.11$^{\#*}$ | 1,696.98 ± 148.94$^{\#*}$ |
| BSA + Fructose + KY 2.00 mg/ml | 829.45 ± 111.90$^{\#*}$ | 1,082.81 ± 32.27$^{\#*}$ | 1,308.21 ± 111.19$^{\#*}$ | 1,506.79 ± 117.23$^{\#*}$ |
| BSA + Fructose + AG 0.50 mg/ml | 2,878.55 ± 40.38 | 3,713.34 ± 311.67 | 4,019.65 ± 111.32 | 4,203.31 ± 147.87 |
| BSA + Fructose + AG 1.00 mg/ml | 2,643.23 ± 45.26 | 3,456.56 ± 367.52 | 3,658.87 ± 132.55 | 3,949.02 ± 101.10 |

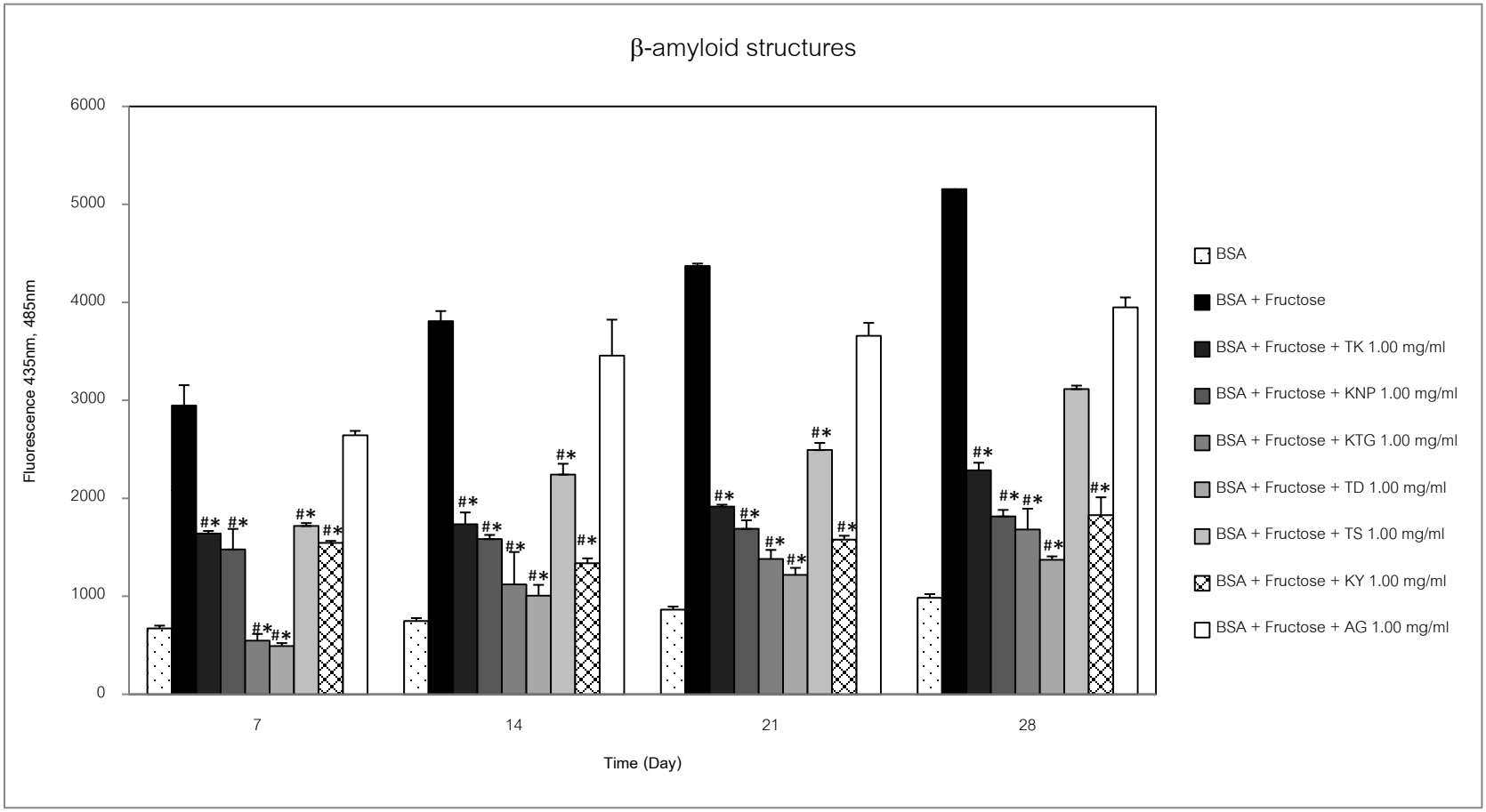
Data are expressed as mean ± SEM, n =3. $^{\#}P < 0.01$ compared to BSA+Fructose and $^{*}P < 0.01$ compared to aminoguanidine 1.00 mg/ml.

**Figure 32** – The effect of pomelo cultivar (Kao Yai) on the formation of β-amyloid structures at day 7, 14, 21 and 28.




Data are expressed as mean ± SEM, n =3. $^{\#}P < 0.01$ compared to BSA+Fructose and * $P < 0.01$ compared to aminoguanidine 1.00 mg/ml.

**Figure 33** - The effect of the pomelo cultivars (1.00 mg/ml) on the formations of β-amyloid structures at day 7, 14, 21 and 28 compared to aminoguanidine 1.00 mg/ml




Data are expressed as mean ± SEM, n =3. $^{\#}P < 0.01$ compared to BSA+Fructose and $^{*}P < 0.01$ compared to aminoguanidine 1.00 mg/ml.

## 2.5. Quantification of $N^{\varepsilon}$-(carboxymethyl) lysine (CML) structures

The quantification of CML structures formed as an AGEs was determined on the 28th day. The results showed that BSA incubated with fructose increased the formation of CML structure indicating a progressive AGE structure accumulation. The level of formed CML structures in BSA incubated with fructose and the pomelo cultivar extracts at the concentration of 0.50 mg/ml were significantly decreased at day 28 of the experiment ($P<0.01$) (Table 40)

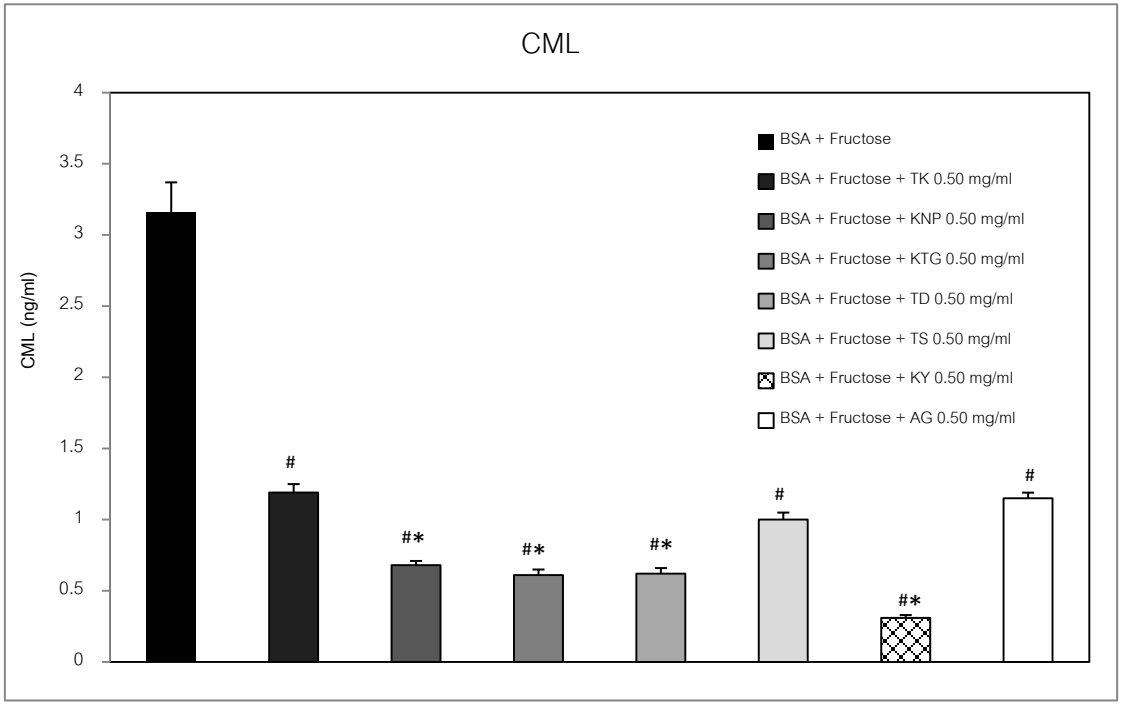
When compared to the BSA + fructose, the pomelo cultivars at 0.50 mg/ml Kao Yai, Kao Tanggwa, Thong Dee, Kao Nam Pueng, Tubtim Siam and Tar Koi were able to decrease the formations of CML structures by 90.19±0.02, 80.70±0.01, 80.38±0.03, 78.48±0.07, 68.35±0.09 and 62.41±0.08%, respectively. The aminoguanidine (0.50 mg/ml) was able to also to decrease the formations of CML by a 63.61±0.01% when compared to the BSA + fructose. Remarkably, the pomelo cultivars Kao Yai, Kao Tanggwa, Thong Dee, Kao Nam Pueng and Tubtim Siam were able to exert a 73.04±0.01, 46.96±0.02, 46.09±0.04, 40.87±0.09, 13.04±0.02% higher amount than that of aminoguanidine, respectively. As for the pomelo cultivar Tar Koi when compared to aminoguanidine (0.50 mg/ml), exhibited a slightly lesser inhibition ability (Figure 34).

**Table 40** - The effect the pomelo cultivars on the formation of CML structures at day 28.

| | The level CML (ng/ml) |
|---|---|
| BSA + Fructose | 3.16 ± 0.21 |
| BSA + Fructose + TK 0.50 mg/ml | 1.19 ± 0.06[#] |
| BSA + Fructose + KNP 0.50 mg/ml | 0.68 ± 0.03[#*] |
| BSA + Fructose + KTG 0.50 mg/ml | 0.61 ± 0.04[#*] |
| BSA + Fructose + TD 0.50 mg/ml | 0.62 ± 0.04[#*] |
| BSA + Fructose + TS 0.50 mg/ml | 1.00 ± 0.05[#] |
| BSA + Fructose + KY 0.50 mg/ml | 0.31 ± 0.02[#*] |
| BSA + Fructose + AG 0.50 mg/ml | 1.15 ± 0.04[#] |

Data are expressed as mean ± SEM, n =3. $^{\#}P < 0.01$ compared to BSA+Fructose and $^{*}P < 0.01$ compared to aminoguanidine 0.50 mg/ml

**Figure 34** - The effect the pomelo cultivars on the formation of CML structures at day 28.




Data are expressed as mean ± SEM, n =3. $^{\#}P < 0.01$ compared to BSA+Fructose and $^{*}P < 0.01$ compared to aminoguanidine 0.50mg/ml

# Chapter 5

## Discussion

Hyperglycemia is one of the primary underlying causes and pathogenesis of long-term complications in diabetes mellitus. Hyperglycemia through the mechanisms of increased polyol pathway, increased hexosamine pathway, activation of protein kinase C, increased formation of products of nonenzymatic glycation and oxidative stress (Lapolla et al., 2005) causes dysfunction of organs and the higher propensity of micro- and macro-vascular diabetic complications. Of which, the pathway of nonenzymatic glycation has received much interest in terms of treatment to prevent complications as evidence. It is suggested that this pathway is one of the major causes to contribute to the glycoxidative reaction and complications. Glycoxidative reaction is an important modulator of the formation of the nonenzymatic glycation that eventually leads to the irreversible formation of AGEs. This event can cause significant modifications to normal cellular functions and structures such as increasing the permeability of vascular cells and the crosslinking of long lived proteins to lead to disparities in protein function (Fowler, 2008; Lalla, 2000). Alterations of such function lead to an accumulation of high oxidative stress and generations of ROS.

Glycation induces the generation of ROS that causes high levels of oxidative stress that occurs as a result of processes of autoxidative glycation and glycoxidation. Autoxidative glycation occurs when the aldehyde groups of monosaccharides, undergoes autoxidation in the presence of transition metal such as iron or copper, to spontaneously reduce molecular oxygen and generate an enediol radical. The radical then reduces oxygen to form the superoxide radical and is further oxidized to a dicarbonyl ketoaldehyde that can further react with protein amino groups to generate a ketoimine (Ahmed, 2005). During glycoxidation, Amadori products are autoxidised to AGEs. In the presence of transition metals, Amadori products are converted into protein dicarbonyl compounds through the protein enediol radical, generating a superoxide radical. Of which the protein

dicarbonyl compound participates in AGE formation and the formed superoxide radicals are further converted to a highly oxidative hydroxyl radical *via* Fenton reaction (Ahmed, 2005).

Numerous effort and interest has been focused on the development of inhibitors of protein AGEs to suppress or to alleviate the complications of hyperglycemia. A known glycation inhibitor, aminoguanidine, has shown effective improvement in diabetic complications, however aminoguanidine is still under clinical trials and has been reported to cause long term adverse effects such as rare vasculitis and kidney tumors (Pashikanti et al., 2010; Thornalley, 2003). Compounds of natural foods or nutrients that inhibit radical formation and suppress the formation of AGEs have great therapeutic importance for the prevention of the pathogenesis (Wu et al., 2009).

Pomelo (*Citrus grandis* Osbeck), a native citrus fruit to South East Asia and one of the largest Citrus species, are widely cultivated and utilized in Thailand. There are many cultivated varieties of the pomelo; however the most common are Kao Yai, Kao Nampueng, Thong Dee, Kao Tanggwa, Tubtim Siam and Tar Koi. Like citrus fruits, pomelos are an abundant source of vitamin C, folic acid, beta carotenoids and limonoids (Sai et al., 2007). Phenolic compounds have also been identified in pomelos such as, caffeic acid, coumaric acid, ferulic acid, sinapic acid, hyrdoxyl-benzoic acid and vanillic acid (Xu et al., 2007). In addition to phenolic compounds, citrus flavonoids have also been identified in the pulp of pomelo such as naringin, narirutin, neohesperidin and kaempferol (Abeysinghe et al., 2007). Specifically in Thai pomelo cultivars, previous findings demonstrate that naringin, hesperidin and neohesperidin, were also found present (Pichaiyongvongdee et al., 2009). Thus, this suggests that the identified flavonoids in the present study are consistent with previous findings; alongside with an additional of flavonoids that were also identified, including of neohesperidin dihydrochalcone, naringenin and hespertin.

Pomelo has also shown potent antioxidant properties. Multiple studies have documented antioxidant activity of pomelo through its ability to inhibit the formation of free radicals generated by 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{+}$) radical and to inhibit the

oxidation of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^{+\bullet}$) (Jayaprakasha et al., 2007). Moreover, the pomelo extracts have also showed abilities in decreasing peroxyl radicals through the oxygen radical absorbance capacity (ORAC) assay (Jayaprakasha et al., 2007).The pomelo extracts have showed a high ability to reduce $Fe^{3+}$ to $Fe^{2+}$, which suggest of its ability to suppress the formation of the Fentron reaction and hence, impeding the formation of a highly reactive hydroxyl radical (Pichaiyongvongdee et al., 2009; Valko et al., 2006). The citrus flavonoids found in pomelo have shown to possess antioxidant activity (Tsai et al., 2007) which is directly related to their ability to inhibit radical formation, anti-lipoperoxidation and chelate transitional metals (Yao et al., 2004), and may perhaps facilitate their abilities to inhibit glycation. For instance, neohesperidin dihydrochalcone and hesperidin have shown the effective ability to inhibit superoxide formation and oxygen species (Suarez et al., 1998). The abilities of flavonoids to stabilize reactive oxygen species is that flavonoids contain phenolic hydroxyl groups which allow the flavonoids to readily donate electrons to the free radicals, resulting in free radicals becoming more stable and hence less reactive. In addition, the presence of the 4-oxo function located in the C ring of flavonoids was also found in the identified flavonoids in the six pomelo cultivars. This leads to ascribe their abilities to chelate transition metals (Nijveldt et al., 2001; Pietta, 2000). For that reason, it is possible that the citrus flavonoids found in pomelo may possess great strength in inhibiting the formation of AGEs by their abilities to inhibit superoxide formation, stabilize free radicals and oxygen species.

In this study, the antiglycation effects of six pomelo cultivar extracts against a fructose induced non enzymatic glycation in bovine serum albumin (BSA) and oxidation-dependent damage to BSA were evaluated. The present experiments had measured the outcomes between specific inhibitors and pomelo extracts during the early stage (Amadori Products), middle stage (protein oxidation) and last stage (AGEs formation and cross-linking) of AGE formation. The formations of AGEs are a diverse class of protein modifications arising from various chemical reactions, including condensations, rearrangements, fragmentations, and

oxidative structural modifications. Primarily, a non-enzymatic reaction between a reducing sugar and the amino groups of proteins occurs which then forms a Schiff base reaction that generates intermediates which undergo further cascade transformations *via* Amadori products, and Malliard reactions leading to the formation of AGEs (Peppa et al., 2005; Peyroux et al., 2006). Fructosamine or an Amadori product is an early glycation product that is form from the formation of a Schiff Base. A Schiff base is formed non enzymatically when fructose reacts with amino group of BSA which then is rearranged into an Amadori product. The formed fructosamine then undergoes complex cascades of dehydration, oxidation, condensation, fragmentation and cyclization reactions (Thome et al., 1997).

A comprehensive previous study related to naturally occurring polyphenols and flavonoids demonstrated that they reduce the formation fructosamine which may correlate their properties of antiglycoxidative activities (Wu et al., 2006). Another study conducted on guava leaf extracts that contained high amounts of polyphenols also showed the suppression of formed fructosamine in BSA by inhibiting scavenge free radicals and possessing metal ion chelative effects (Wu et al., 2009). Of which, the present observations are consistent with the previous studies regarding pomelo extract's ability to decrease the formation of fructosamine. The reductions of fructosamine thereby decrease the further formation of protein oxidation and possibly formations of AGEs. It is possible to indicate that the pomelo extracts reduce the formation of fructosamine due to of the antiglycoxidative activities *via* its ability to chelate metal ions, scavenge free radicals, leading to reduce the covalent attachment of glucose to BSA.

The reactive carbonyl intermediates are formed by the process of protein oxidation, a process arose by the formation of the Schiff base that is highly prone to oxidation and free radical generation (Valko et al., 2006). As a result of the oxidation of the Schiff base and the excess of free radicals, the Amadori product is oxidized and in turn causes the formation of reactive carbonyl intermediaries and ROS, precursors to the formation of AGEs ( Wu et al., 2011). The formed carbonyl intermediaries have the ability react with protein to result in the denaturation, browning and crosslinking of proteins

causing the formation of protein carbonyl derivatives and then the formation of AGEs, causing severe and irreversible oxidative damage to the protein (Goldin et al., 2006; Dalle-donne et al., 2003). Hence, the overproduction ROS and formation of reactive carbonyl intermediaries are important biomarkers of severe protein oxidative damage and protein modification.

Aside from the formation of AGEs, reactive carbonyl intermediaries and protein carbonyl derivatives also cause modifications to proteins, especially proteins that are particularly prone to oxidative attacks to amino acid such a cysteine. Cysteine is a vital component to maintain the protein's structure, enzymatic activity and antioxidant activity. However, it can be destroyed by the oxidation, resulting in the release of free thiol groups (Dalle-donne et al., 2003). The oxidation of thiol groups results in conformational changes in protein structure, creating disulfide bonds, and high levels of generated free radicals (Murphy et al.,1989). Thus, the increased levels of oxidized thiol groups are reflective of high oxidative stress, protein oxidative damage and formation of AGEs.

Subsequent with the occurrence of oxidized proteins are the formation of cross β-amyloid structures in the albumin, which are also formed *via* reactive carbonyl intermediaries. Cross β-amyloid structures form as a result of irreversible cross-linking of heterogeneous protein aggregates. This causes the protein albumin to form a beta sheet conformation and the occurrence of protease-resistant and ubiquitin-proteasome-resistance derived from the formation of AGEs, causing modifications to protein function and accelerated degradation of the protein albumin (Wu et al., 2011; Vitek et al., 1994). The cross β-amyloid structures can also cause disruption to the native state of the protein albumin by denaturation (Kikuchi et al., 2003). The denaturation of albumin causes protein ubiquitination that leads accelerated protein destruction, denatured albumin to interact inappropriately to with other cellular components leading to cellular impairment altered enzyme activity and explosion of specific sites like that hydrophobic sites that are normally inaccessible in the native form of albumin (Rondeau et al., 2011). Alongside with oxidation

processes, the cross β-amyloid structures generate insoluble β-amyloid plaques and β-sheet fibrils that can cause toxicity to neuronal cells (Deuther-conrad et al., 2001).

The pomelo extracts consist of high amounts of neohesperidin, heperidin, naringin and naringenin which all structures contain of a hydroxyl group at C-3' position on the B ring allowing for these flavonoids to be able scavenge oxygen species. Similarly, a study also shows that citrus flavonoids have the abilities of reduction of carbonyl formation *via* the high antioxidant properties of citrus flavonoids (Amful et al., 2010). Hesperidin, a citrus flavonoids that also showed abilities to restore levels plasma thiol groups in a protein oxidative damage environment (Ibrahim, 2008). Various studies have also shown that a vast array polyphenols are able to inhibit the formation of cross β-amyloid structure not only through the scavenging of free radicals but also by directly blocking and inhibiting the deposits of fibrillar β-amyloid (Porat et al., 2006). In this present study, the pomelo extract that contains high levels various levels of flavonoids also showed abilities in reducing levels of protein oxidation and protection of thiol group. Hence, the pomelo extracts ability to increase the levels of thiol groups, indicate that the pomelo extract seemingly acquires the ability to decrease the formation of disulfide bonds that occur during protein oxidation and thus, maintain the protein structure. In combination with suppression of protein carbonyl content and increase of thiol group levels, the pomelo extracts were also able to decrease levels of cross β-amyloid structure formations. Accordingly, the pomelo extract and its flavonoid composition are probable of alleviating protein oxidation reflected by its capacity to scavenge free radicals and to restore protein structure.

CML is one of an AGE structure that is formed between a reactive carbonyl intermediate and an amino acid residue during the formation of a Schiff base. Amplified by glycoxidation, the Schiff base undergoes oxidative cleavage to form a CML structure (Reddy et al., 1995). The CML structure is known to be the most abundant AGE structure formed and its accumulation can be enhanced under conditions of chronic hyperglycemia (Fu et al., 1996). Multiple studies have also revealed that the CML structure is closely

related to the pathogenesis of the diabetes complication, retinopathy and nephropathy (Monnier et al., 2005).

In unification with flavonoids abilities to scavenge free radicals and chelate transitional metals, flavonoids have continuously showed abilities in reducing formations of AGEs (Saraswat et al., 2007; Urios, 2007). A study conducted on a variety of flavonoids showed that the inhibitory activity on the formation of pentosidine, one of an AGE structure. It has been reported that this inhibitory activity is due to its abliltiy to chelate transition metal ions, hence preventing the metal-catalysed formation of hydroxyl derived radicals (Urios, 2007).The pomelo extract, also containing various amounts of flavonoids that although the amount of flavonoids did not correlate to the amount of inhibition to each pomelo cultivar, in this study showed inhibition of AGEs in a similar manner. AGEs are largely composed of two types; the fluorescent cross-linking type and the non cross-linking non fluorescent type. The pomelo extracts showed potent suppression in both types of AGE formation. In particular, the pomelo extracts were able to decrease formation of a CML structure, indicating that the pomelo extract attains potent abilities of suppressing in the overall formation of AGEs, the last stage of protein glycation. Also, attributable to the pomelo extract's ability to reduce the formation of fructosamines, an early glycation product, may have also contribute to the reduction of AGE formation.

# Chapter 6

## Conclusion

This study demonstrates that the six pomelo cultivars (Tar Koi, Kao Nam Pueng, Kao Tanggwa, Thong Dee, Tubtim Siam and Kao Yai) contains of various flavonoids which exhibits strong antiglycation actions. The pomelo cultivar that contains the highest amounts of flavonoids was found to be Tar Koi. With great inhibitory effects of glycation, these pomelo cultivars may potentially be developed in the form a nutraceutical food that is cost effective and widely accessible. The extracts can also be applied as medically directed functional food to minimize and ameliorate the development of protein glycation-induced diabetic complications. The development and commercialization of the extracts can not only hold great health benefits but also economical benefits and that it is advantageous to indigenous communities as well as to the health of the general population.

In spite of the pomelo extracts showing favorable antiglycation properties, these effects were able only displayed in *in vitro* model. To further investigate the antiglycation effects of pomelo, *in vivo* studies should be conducted in animal models and in diabetic patients to determine the efficacy and of any toxicological effects.

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# Biography

Natarin Caengprasath was born in Bangkok, Thailand. After graduating from Bangkok Patana School in 2006, she entered Chulalongkorn University as an applied chemistry major, with an emphasis in industrial management. From her completion of her senior project entitled ‘Addition of omega- 3 fatty acids to snacks for increased nutrition value’, she has grown a fawn interest for nutrition. Directly from obtaining her BSc in 2009, she entered the Graduate School of Nutrition and Dietetics at the Allied Health Science faculty at Chulalongkorn University and majored in Medical Nutrition Therapy.