APPLICATION OF ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES OF CHIANG RAI ASSAM GREEN TEA INFUSION IN FOOD MODEL SYSTEM

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MASTER OF SCIENCE

IN FOOD TECHNOLOGY

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A THESIS SUBMITTED TO
MAE FAH LUANG UNIVERSITY IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
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Risky Ayu Kristanti

Thesis Title Application of antioxidant and antimicrobial properties of Chiang Rai assam green tea infusion in food model system

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ABSTRACT

Recently, green tea has received much attention and popularity in worldwide consumption as a result of its health benefits. Those health benefits are considered to be its high antioxidant and antimicrobial properties. Chiang Rai is the biggest tea cultivation area in Thailand. However, tea antioxidant and antimicrobial properties are influenced by various factors, including the cultivating area, cultivating practise and processing. Therefore this study aimed to investigate the antioxidant and antimicrobial properties of Chiang Rai's commercial assam green tea infusion *in vitro* and the ability of those commercial assam green tea infusions to extend shelf life of some food models.

The investigation of antioxidant properties of commercial assam green tea infusion in Chiang Rai, represented by *A, B, C, D,* and *E,* were evaluated *in vitro* in terms of total polyphenol content (TPC) and diphenyl-picrylhydrazyl (DPPH) scavenging activity. Whereas, antimicrobial activity was evaluated against 4 selected pathogens, *Staphylococcus aureus*, *Salmonella typhimurium*, *Escherichia coli* and *Listeria monocytogenes*, tested by an agar diffusion method. Some chemical composition profiles in green tea products were evaluated by HPLC. It was found that *A* provided the highest TPC (23.50 % w/w dry basis (db)), followed by *B* (20.18 % w/w db), *C* (17.40 % w/w db), *D* (15.95 % w/w db) and *E* (15.86 % w/w db), respectively. These results were consistent to their DPPH scavenging activity and their pathogen inhibitory ability. All green tea infusions were notably able to inhibit gram-positive bacteria than

gram-negative bacteria. The main compounds found in all assam green tea infusions were caffeine (CF), epigallocatechin (EGC), epicathechin (EC), gallic acid (G) and catechin (C). Assam green tea infusion with the highest antioxidant and antimicrobial activities contained both EC and EGC as major catechins. These suggest that these EC and EGC contributed to their antioxidant and antimicrobial activities.

Assam green tea infusions were also applied to liquid medium containing the selected microorganisms to evaluate their ability to inhibit microorganisms to mimic liquid food models. Minimum Inhibitory Concentration (MIC), which is the tea concentration exhibiting completely inhibitory effects, was used to determine the assam green tea infusion microbial inhibitory effect in this experiment. Among all assam green tea infusions, *A* and *B* were observed to have the most inhibitory effect to all pathogens in the liquid medium, indicated by the the lowest MICs for *S. aureus* (50 mg/ml)*, L. monocytogenes* (75 mg/ml), *S. typhimurium* (150 mg/ml) and *E. coli* (225 mg/ml), compared to *C, D* and *E.* These results were also observed when the assam green tea infusion was applied to watermelon juice incubating with the selected microorganisms at 35 °C for 7 days. The results showed that the pH of watermelon juice was significantly reduced when the assam green tea infusion was added ($p \leq 0.05$). *A* and *B* inactivated *S. aureus, L. monocytogenes, S. typhimurium* and *E. coli* within 2, 3, 5 and 6 days, respectively.

Assam green tea infusion was also applied to cooked meat model systems in order to evaluate its anti-lipid oxidation and antimicrobial activities. *A* and *B* at the concentration of 250 mg/ml effectively reduced all pathogens in the cooked beef to an undetectable level within 2 days of storage at 4 °C. It was also found that *A* and *B* exhibited higher anti-lipid oxidation activity compared to the commercial antioxidant, 0.02 % BHA/BHT and the control, respectively. The addition of those assam green tea infusions in cooked beef significantly increased ΔL^* value and decreased Δa^* and Δb^* value ($p \leq 0.05$). These indicate that Chiang Rai assam green tea infusion provided significant improvements in terms of the microbial safety and quality of watermelon juice and cooked beef, and it might be a good preservative for preserving other types of food with the same purposes.

Keywords : Antimicrobial, Antioxidant, Assam green tea, Cooked beef, Shelf life, Watermelon juic

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CHAPTER 1

INTRODUCTION

1.1 Background

The oxidation of lipid and microbial contamination in food products are two of the major causes of food deterioration (Pearson *et al.,* 1977; Adams and Moss, 2000). These result in food quality and shelf life reduction, rendering the product unacceptable for consumption. Therefore, various means have been applied in order to retain those qualities of fresh-like products and extend shelf life, while assuring the product safety. Those means include heat processing, irradiation, low temperature storage, high hydrostatic pressure, high intensity ultrasound as well as the addition of preservatives (Odumeru, 2006). The preservative addition is one of the efficient methods providing those characteristics; however, some limitations have been encountered due to their health risks (Buxiang and Fukuhara, 1997; Hirose et al., 1998). As a result of increasing health concern of consumers as well as the demand of fresh-like products with maintained nutritional and sensorial integrity, efforts have been made in order to find out some potent natural preservatives to maintain food quality and assure food safety (Madsen and Bertelsen, 1995; McCarthhy et al., 2001; Rhee et al., 2001, Lemay et al., 2002).

In the past few years, various natural preservatives have been used in foods, such as essential oils, plant extracts, tocopherol and organic acids (citric, polylactic and acetic acid) (Tang et al., 2001; Lemay et al., 2002; Min-Suk et al., 2003; Mosqueda-Melgar et al., 2007). Those preservatives have shown to effectively inhibit oxidation, microbial contamination and multipication in many kinds of food, such as hamburgers, unpasteurized fruit juice, sausages, hot dogs and meatballs (Fernandez-Lopez et al., 2005; Riznar et al., 2006; Mosqueda-Melgar et al., 2007; Busatta et al., 2008).

Green tea extract is one of the potent natural preservatives applied to many foods as antioxidant and antimicrobial agents (Wang and Zhao, 1997; Tang et al., 2001; Almajano et al., 2008). Their antioxidant and antimicrobial properties are responsible by their rich polyphenols content (Wang et al., 2000; Zheng and Wang, 2001; Tiwari et al., 2005; Khan and Mukhtar, 2007; Almajano et al., 2008). The polyphenols found in green tea are mostly catechins, such as catechin (C), epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG) (Wang et al., 2000; Zhen, 2002). It has been reported that tea catechins have antioxidant activity that is ten times higher than *L*-ascorbate and *β*-carotene (Nakao et al., 1998). Other studies have also demonstrated antimicrobial activity against some potent pathogens, such as *Shigella dysenteriae, Salmonella* sp*, Eschericia coli, Staphylococcus aureus* and *Listeria monocytogenes* (Cheng-Chun et al., 1999; Wang et al., 2000; Sakanaka et al., 2000).

Even though, several studies have been conducted *in vitro* on the antioxidant and antimicrobial properties of green tea extract, many factors have been reported to affect these properties as those properties from other plant materials (Chan et al., 2008; Hussain et al., 2008). The factors include tea variety, cultivation area, cultivation practise, season of harvesting, processing and extraction method (Cheng-Chun et al., 1999; Zhen, 2002). In particular, the processing method has many variables impairing the properties, such as drying temperature and time (Uzunalic et al., 2006).

Chiang Rai is the province with the largest assam green tea cultivation in Thailand (Anonymous, 2008). Various products from different vendors are available in local markets. Until now, there is no report on the antioxidant and antimicrobial properties of commercial assam green tea available in Chiang Rai. In addition, there are only a few studies investigating the assam green tea infusion antioxidant and antimicrobial properties in foods (Wang et al., 2000; Yilmaz, 2006). Therefore, this work is aimed to investigate antioxidant and antimicrobial properties of commercial assam green tea infusion *in vitro* and in some food models. These will provide basic information of the commercial assam green tea infusion in Chiang Rai, which might further benefit other researchers as well as consumers.

This present chapter provides a summary of relevant background information, the objectives, the scope, the significance and the thesis outline.

1.2 Objectives

The objectives of this study are :

1.2.1 To investigate the antioxidant and antimicrobial properties of Chiang Rai's commercial assam green tea infusion *in vitro*

1.2.2 To investigate the ability of Chiang Rai's commercial assam green tea infusion to extend the shelf life of some food models

1.3 Scope

In order to achieve the main objectives of this work, three approaches were performed as shown below:

1.3.1 Investigation of antioxidant and antimicrobial properties of commercial assam green tea infusion *in vitro* by DPPH scavenging activity and the agar diffusion method

1.3.2 Investigation of antimicrobial activity of commercial assam green tea infusion in liquid medium to mimic the liquid food model using the macrodillution broth method

1.3.3 Application of commercial assam green tea infusion as an antioxidant and antimicrobial in some food models; watermelon juice and cooked beef

1.4 Thesis Significance

This research provides new information about antioxidant and antimicrobial properties of commercial assam green tea infusion *in vitro* and in some food models, both liquid and solid. These properties could be further exploited in various types of food.

1.5 Thesis Outline

This thesis is focused on investigating the antioxidant and antimicrobial activities from Chiang Rai's commercial assam green tea infusion, and their ability to extend shelf life in food. The thesis is composed of six Chapters with the content described in brief as follows;

The first chapter provides a summary of relevant background information, the objectives, the scope of work, the significance of work and the thesis outline.

The second chapter states, reviews and theorizes on green tea, tea composition, antioxidant and antimicrobial properties to explore further application purposes and the potential of green tea in food.

Chapter three describes the antioxidant and antimicrobial activities of commercial assam green tea infusion in Chiang Rai (*in vitro*) in order to further application purposes of these material.

Chapter four and five explain the antimicrobial activity of commercial assam green tea infusion in liquid medium and watermelon juice, respectively.

Chapter six illustrates survival of pathogenic bacteria in cooked beef containing commercial assam green tea infusion as well as their effect on lipid oxidation in cooked beef.

Chapter seven concludes all the results from the performed work and provides some recommendations.

CHAPTER 2

LITERATURE REVIEWS

2.1 Introduction

Tea is one of the most popular beverages consumed throughout the world. Tea is consumed as green (unfermented), oolong (semifermented) and black (fermented) tea. In recent years, tea has been more attractive because of its health benefits. It has been reported that tea reduces blood pressure, cholesterol, heart attacks, stroke and cancer as well as enhances digestion and the immune system (Dufreshne and Farnworth, 2001). It also can enhance food stability by inhibiting some microbial growth and inactivate free radical species (Tang et al., 2006; Ishii et al., 2008). It is believed that tea properties, including antioxidant, antimicrobial, anticarcinogenic and antiarterioschelerotic, are responsible for these actions (Wang et al., 2000). These properties offer the possibilities to apply tea as an natural antioxidant and antimicrobial in foods which comply to the recent health concern food market.

This chapter provides information of tea varieties, tea chemical compositions, factors affecting tea chemical composition, tea health benefits, tea antioxidant and antimicrobial action, and their applications in foods.

2.2 Tea Varieties

Tea, *Camellia sinensis* (L), is an evergreen tree belong to Theaceae family (Weisburger, 1997). There are 3 varieties of tea, including Assamica, Chinese and Cambodian, as described in detail below (Bezbaruah, 1999):

2.1.1 Assamica Tea

The Assamica tea, called *Camellia sinensis* var. *assamica* or *Camellia assamica,* is normally grown in northeast India, Myanmar, Vietnam, and south China (Ming, 1992). This variety grows to a small tree with a vertical branch system and a distinct trunk with the diameter of about one-third of the height of the tree. The tree generally grows to a height of 10-15 m. Assamica tea leaves are more than 100 mm in length, typically dependant, thin, glossy with more or less acuminate apex, and distinct marginal veins (Jain, 1999). The flowers are yellow-white, 2.5–4 cm in diameter with 5 petals and a light green pistil. The morphology of Assamica tea leaves is shown in Figure 2.1.

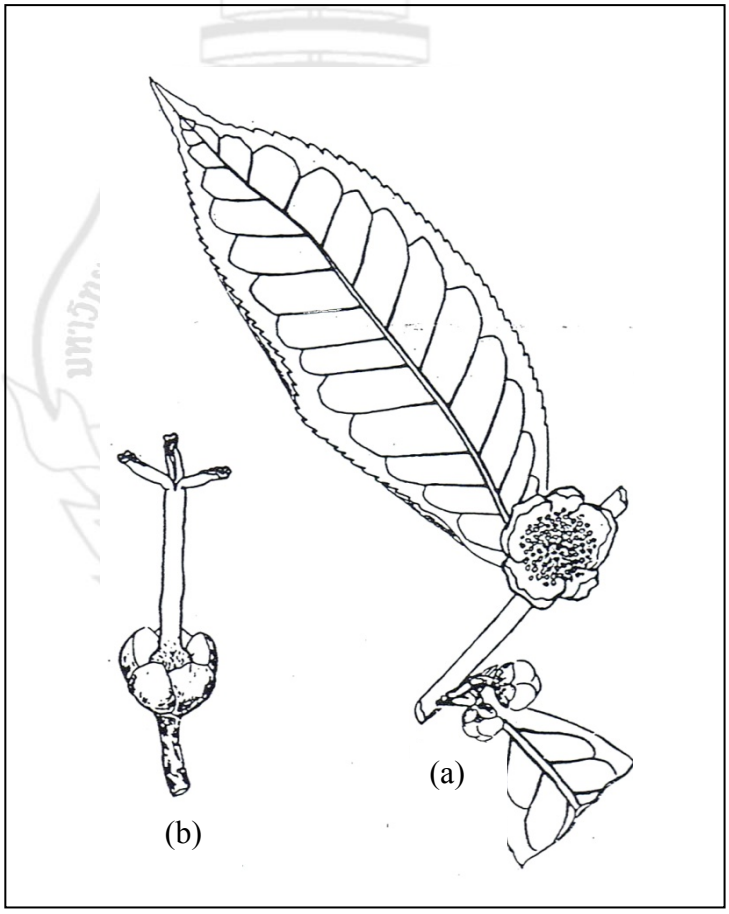


Figure 2.1 Assamica variety tea (a) flowering shoot (natural size); (b) pistil (4x magnification)
Source : Bezbaruah (1999)

2.1.2 Chinese Tea

The Chinese tea, called *Camellia sinensis* var *sinensis,* is naturally found in southeast China (Ming, 1992). This variety grows to a big shrub of about 1-3 m height with numerous virgate stems arising from the base of the bush near the ground level. These give a dome shaped bush when the tree is completely grown. Chinese tea leaves are smaller than Assamica (60-100 mm in length), erect, small, and characteristically thick (Jain, 1999). The flower is smaller than Assamica. It has multiple yellow stamen and a light green pistil. The fruits are green in colour with 2–3 seeds, and it starts bearing within 5–6 years after planting. The morphology of Chinese tea is shown in Figure 2.2 (Bezbaruah, 1999).

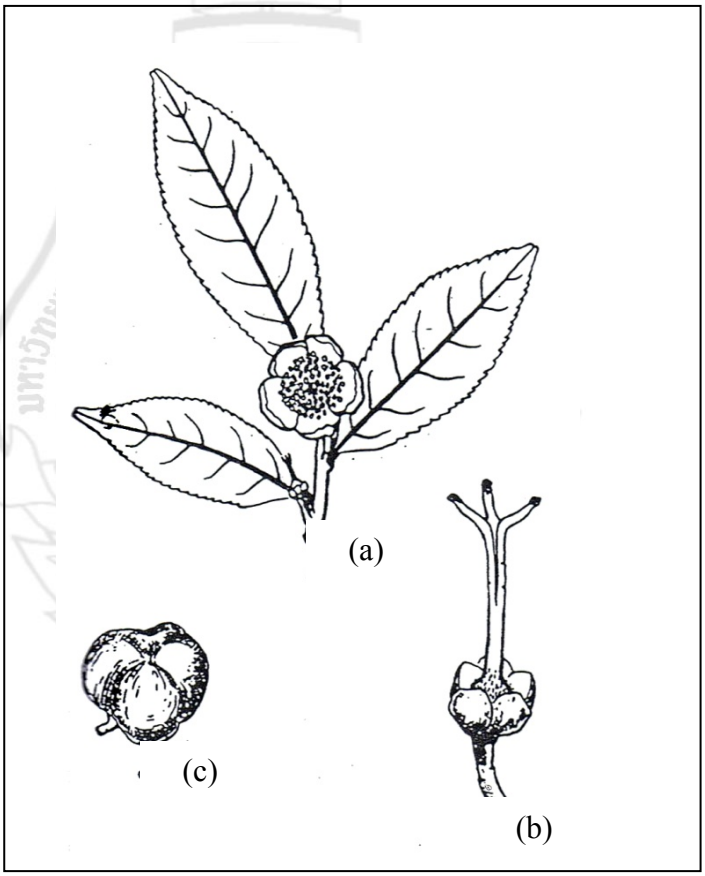


Figure 2.2 Chinese variety tea (a) flowering shoot (natural size); (b) pistil (4x magnification); (c) fruit (natural size)

Source : Bezbaruah (1999)

2.1.3 Cambodian Tea

The Cambodian tea, called *Camellia sinensis* var *lasiocalyx*, is referred to as a hybrid of the Assamica and Chinese tea (Ming, 1992). This variety is a fastigate tree with more or less equally developed ascending main stems, reaching a height of about 6-8 m. Cambodian tea leaves range in size between that of Assam and Chinese tea, and are more or less erect and glossy. It is yellowish-green when it is young, while light green at its maturity and becomes coppery-yellow or pinkish-red during autumn (Jain, 1999). The morphology of Cambodian tea is shown in Figure 2.3 (Bezbaruah, 1999).

The Assamica tea is the most cultivated of tea because it can grow well in the tropical region. If compared to Chinese tea, Assamica tea gives higher yield, while the Chinese tea has more delicate flavor. Chinese tea is able to grow well at high altitudes (Arifin and Semangun, 1999).

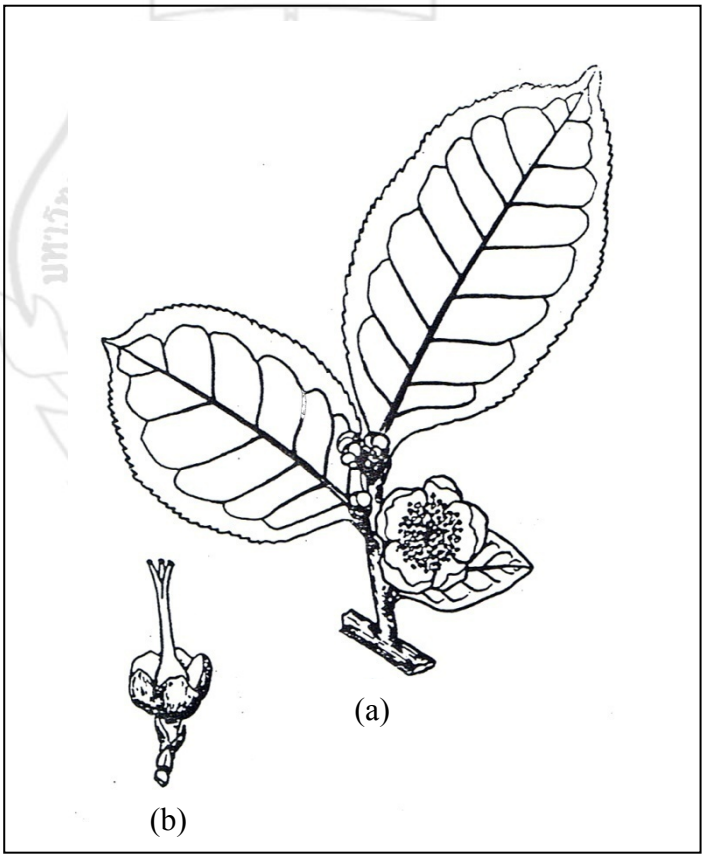


Figure 2.3 Cambodian variety tea (a) flowering shoot (natural size) (b) pistil (4x magnification)
Source : Bezbaruah (1999)

According to statistics from the Food and Agricultural Organization (FAO, 2006) of the United Nations, production and consumption of tea are increasing every year (Table 2.1 and 2.2). The main tea-producing countries are China (26.68 %), India (26.49%), Kenya (9.38%), Sri Lanka (9.05 %), Turkey (5.87 %), Indonesia (4.73%) and Vietnam (2.97 %).

As a result of the increasing tea consumption, the tea supply is not enough for the tea market. Therefore, many countries including Thailand, have been attempting to extend the tea plantation area.

Table 2.1 World tea production

Location	Tea production (thousand tons)				
	2001	2002	2003	2004	2005
World	3046	3173.7	3249.3	3387.9	3503.7
China	722	765.7	791	856.2	934.9
India	856	883.0	907	893	928
Kenya	295	287.1	293.7	324.6	328.5
Sri Lanka	296	310.6	303.2	308.2	317.2
Turkey	143	150	155	205.6	205.6
Indonesia	173	172.8	167.5	169.8	165.8
Vietnam	81.7	93	94.5	97	104

Source : FAO, 2006

The Thailand tea cultivation area is located in Chiang Rai, Chiang Mai, Nan, Phrae, Lampang, Tak and Mae Hong Son, with a total production area of 118,100 rai (18,896 hectares) (Noppakoonwong and Nillavesana, 1999). Half of the total tea cultivation area, about 41,337 rai (6,613 hectares), is located in Chiang Rai with the spectacular scenary as shown in Figure 2.4.

In 2008, Thailand produced 61,557 tons of fresh tea leaves (Anonymous, 2008). The produced fresh tea leaves (about 77 %) was processed to dried tea leaves, while the rest (23 %) was used to produce traditional fermented tea (Miang). Only Assamica and Chinese are cultivated in Thailand due to preferable characteristic with the production area of 15,767 hectare (84.4 %) and 3,129 hectares (16.6 %), respectively (Anonymous, 2008).

Table 2.2 World tea consumption

Location	Tea consumption (thousand tons)					
	1996-2000	2001	2002	2003	2004	2005
World	2833.4	2985.4	3092.6	3199.1	3227.2	3361.6
China	635.4	671.3	693	714	735.0	757
India	482.0	496.2	537.8	555.3	603.7	675.3
Kenya	145.6	156	166.1	168.6	169.1	180.3
Sri Lanka	138.1	149.1	134.9	138.2	156	150.2
Turkey	108.6	106.8	99.4	118.3	120	134.1
Indonesia	142.2	136.7	134.2	119.3	127.8	128.2
Vietnam	91	96.7	93.5	94.1	99.5	100.1

Source : FAO, 2006

Figure 2.4 Tea cultivation in Mae Salong, Chiang Rai (1,200 meters above sea level)

Source : imaged by Punbusayakul, N. in 2007

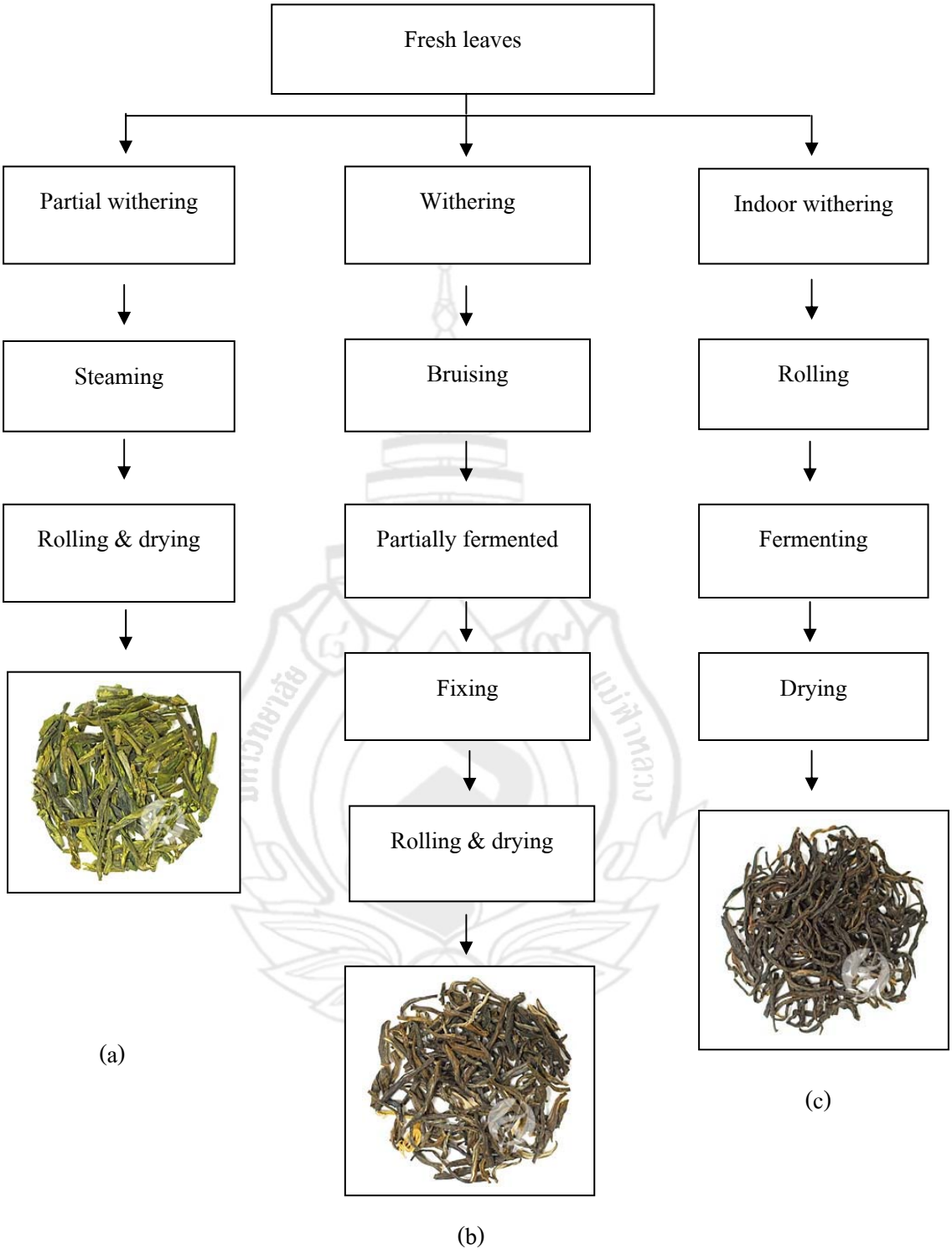


Figure 2.5 The manufacturing process of (a) green tea, (b) oolong tea and (c) black tea

Source : Ho et al. (2009)

Tea products can be classified into 3 types based on the processing; green tea, oolong tea and black tea (Ho et al., 2009). Figure 2.5 shows the processing of tea products. Those basic three kinds of tea have different characteristics, including color, aroma, taste, and appearance. Green tea is produced by steaming and drying the fresh leaves after plucking (Figure 2.5 a). It is also known as unfermented tea due to the polyphenol oxidase enyzme inactivated by steaming. Oolong tea is produced by withering and half fermenting the leaves (Figure 2.5 b). Therefore, oolong tea is called semi-fermented tea. The leaves of black tea (or fermented tea) are completely fermented, allowing enyzmatic oxidation of the polyphenols in tea leaves completely occur as shown in Figure 2.5 c.

Thailand exported 5,394 tons of dried and tea products. The total value was approximately 9.1 million USD. The major export markets include the United States, Taiwan and Cambodia (Anonymous, 2008).

2.3 Tea Chemical Composition

The chemical composition of fresh young tea leaves are complex (Dufreshne and Fanworth, 2001; Zhen, 2002; Hamid, 2006). Polyphenols is the compound mostly found in fresh tea leaves of about 20-40% dry weight, while only trace amounts of other compounds are caffeine, amino acids, organic acids, minerals and vitamins as shown in Table 2.3. Polyphenols are main phenolic compounds in fresh young tea leaves, which are mainly responsible for the unique character of tea when it is processed (Wang et al., 2000; Hamid, 2006). The predominant polyphenols in tea are catechins, which are presented in tea leaves about 18-30% dry weight (Zhen, 2002). Other polyphenols include anthocyanins, flavonols and their glycosides, flavones, proanthocyanidins and phenolic acids (gallic acid and chlorogenic acid) (Ruan, 2005).

Tea contains 7-9% of methylxanthines. This includes caffeine (1,3,7-trimethylxanthine), theobromine (3,7-dimethylxanthine) and theophylline (1,3-dimethyl-xanthine) (Table 2.3). Caffeine is the major methylxanthines. Its content in young tea shoots ranges from 3 to 5%. The reaction of caffeine and other polyphenols such as catechins, theaflavins and thearubigins, can improve its bitter tastes. The quality of tea was correlated to the concentration of caffeine, which contributed to the briskness of black tea infusion (Hamid, 2006).

2.3 Chemical composition of fresh young tea leaves

Components	% Dry Weight
Soluble in water	
Polyphenols	**20-40**
Flavanols	**18-32**
Epigallocatechin gallate (EGCG)	9-14
Epigallocatechin (EGC)	4-7
Epicatechin gallate (ECG)	2-4
Epicatechin (EC)	1-3
Gallocatechin (GC)	1-2
Catechin (C)	0.5-1
Flavonols	1-3
Other flavanoids	5-10
Methylxanthines	**7-9**
Caffeine	3-5
Theobromine	0.05-0.1
Theophyline	0.01-0.03
Amino acids	**5-9**
Theanine	4-6
Others	1-2
Organic acids	**5-9**
Chlorogenic acid	1-3
Gallic acid	0.5-1
Carbohydrates	**3-5**
Minerals	**2-4**
Vitamins (Vit. C, vit. E and vit. K)	**0.6-1**
Volatiles	**0.01-0.02**
Insoluble in water	**15-30**
Cellulose	6-8
Lignin	4-6
Lipid	2-4
Polysaccharide	4-10

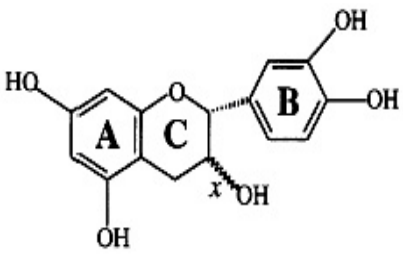
Source : Modified from Dufreshne and Fanworth, 2001; Zhen, 2002; Hamid, 2006

Amino acid also contributed to the tea quality. During the heating process, amino acids, especially theanine, may react with sugars to form furan, pyrazine and pyrrole, which contributed to the roasted aroma of green tea (Dufreshne and Fanworth, 2001). Organic acids, including gallic acid, chlorogenic acid and isoferulic acid, are presented in tea leaves with about 5-9% dry weight (Zhen, 2002). The role of organic acids on the quality of tea is not yet reported (Jayabalan, 2007). Cellulose and lignin in fresh tea leaves contributed to the tenderness of tea material before processing (Zhen, 2002). Polysaccharides in fresh tea leaves are good blood-glucose depressing agents and are recommended for use in the treatment of diabetes (Wang et al., 2000).

All tea product characteristics, including color, taste and aroma are associated with the catechins (Hamid, 2006). For instance, degalloation from ester catechins to non-ester catechins can result in a decrease in the bitterness and astringency of green tea. Other polyphenols, such as flavonols and their glycosides, gallic acid, chlorogenic acid, did not influence the color but was linked to the taste of astringency (Ruan, 2005).

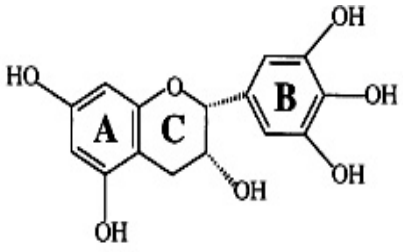
Catechins are colourless and water-soluble compounds which impart bitterness and astringency to tea infusion (Wang et al., 2000). Catechins compose of two aromatic benzene rings (A and B) with three carbon atoms to form an oxygenated heterocycle (ring C) as shown in Figure 2.6. Based on the conformation of the heterocyclic oxygen ring of the molecule, tea leaves contain six major catechins, including catechin (C), epicatechin (EC), gallocatechin (GC), epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) (Figure 2.6). EC has an ortho-dihydroxyl group in the B-ring at C 3’ and 4’ and a hydroxyl group at C 3 on the C-ring. EGC has three hydroxyl groups at C 3’, 4’, and 5’ on the B-ring. ECG has gallate moiety esterified at C 3 of the C-ring. EGCG has three hydroxyl groups at C 3’, 4’, and 5’ on the B-ring and a gallate moiety esterified at C 3 on the C-ring. Among all catechins, EGCG is the most abundant catechins in tea leaves, followed by EGC, ECG and EC (Yilmaz, 2006).

The stability of catechins depends on the number of hydroxyl groups on the B ring (e.g., EC > EGC and ECG > EGCG), the presence of galloyl moiety (e.g., ECG > EC and EGCG > EGC) and the stereochemical structure of each catechin (e.g., ECG > CG and EGCG > GCG) (Kajiya et al., 2004).



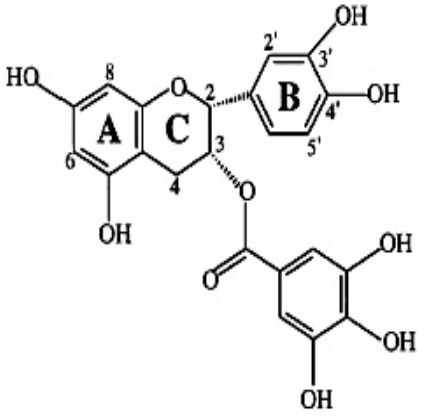
Catechin (C)

(a)



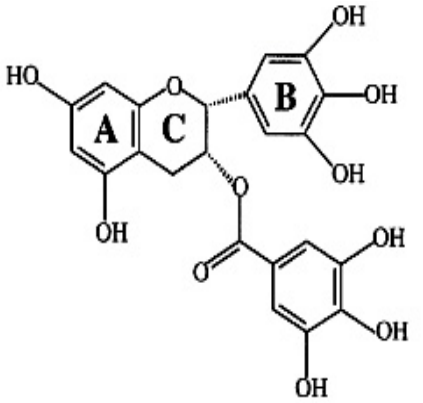
Epigallocatechin (EGC)

(b)



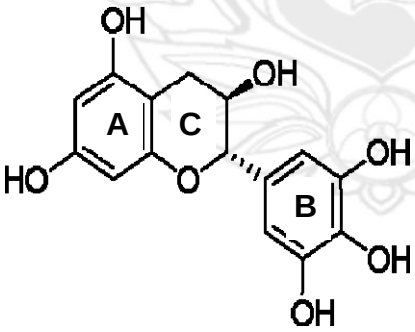
Epicatechin gallate (ECG)

(c)



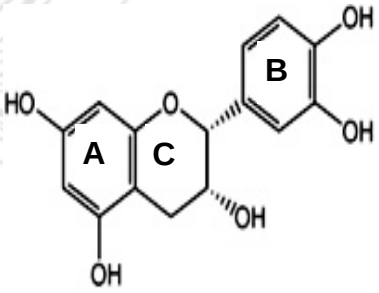
Epigallocatechin gallate (EGCG)

(d)



Gallocatechin (GC)

(e)



Epicatechin (EC)

(f)

Figure 2.6 Chemical structure of major polyphenolic catechins presented in tea (a) catechin (C), (b) epigallocatechin (EGC), (c) epicatechin gallate (ECG), d) epigallocatechin gallate (EGCG), (e) gallocatechin (GC), and (f) epicatechin (EC)

Source : Nurulain (2005)

Polyphenols compounds are found in various fruits and vegetables in different forms as shown in Table 2.4. Tea catechins has the higher polyphenols than others polyphenols such as anthocyanin, flavanol, *p*-hydroxybenzoic acid, hydroxycinnamic acid, myricetin (Arts and Hollman, 2000; Barberan and Clifford, 2000; Daglia et al., 2000; Manach et al., 2004). The amount of polyphenols in tea is about ten times higher than fruit and vegetables, including carrot, tomato, strawberry, apple, etc (Yilmaz et al., 2006). As a higher source of polyphenols, tea has been promoted to enhance human health.

Table 2.4 Polyphenols in fruits and vegetables

Sources	**Form**	**Amount of compound (mg/kg)**	**References**
Carrot	Anthocyanin	11 – 76	Manach et al., 2004
Tomato	Flavanol	2 – 15	Arts and Hollman, 2000
Strawberry	*p*-Hydroxybenzoic acid	20 – 90	Barberan and Clifford, 2000
Potato	Hydroxycinnamic acid	100 – 190	Barberan and Clifford, 2000
Apple	Myricetin	20 – 40	Manach et al., 2004
Coffee	Chlorogenic acid	100 – 120	Daglia et al., 2000
Tea	Catechins	100 – 800	Manach et al., 2004

2.4 Factors Affecting Tea Chemical Composition

Tea chemical composition is affected by various factors, including agro-ecologicals, processing and methods of extraction (Lin et al., 1996; Hamid, 2006; Wang et al., 2000). Investigations on the effect of agro-ecological factors, including tea variety, age of leaves, cultivation area and collecting season in tea chemical composition, had been conducted by several researchers (Takeda, 1994; Lin et al., 1996*;* Cabrera et al., 2006; Farhoosh et al., 2007; Suteerapataranon *et al.,* 2009). The Assamica tea had higher polyphenols content compared to that of the Chinese tea (Cabrera et al., 2006). The higher content of caffeine and tannin were also

observed in Assamica tea (Takeda, 1994; Suteerapataranon et al., 2009). Young leaves (apical bud and two youngest leaves) had higher polyphenols than the older leaves (the tenth to the fifth leaves) (Lin et al., 1996; Farhoosh et al., 2007; Chan et al., 2008). In addition, it was reported that the total polyphenols content of lowland leaves were comparable to that of highland leaves (Farhoosh et al., 2007). Lin et al. (1996) reported that the content of polyphenols in tea leaves harvested in the summer season was higher than the tea leaves harvested in the spring. Cheng-Chun et al. (1999) reported that the average of polyphenols content in tea leaves were 22.0, 20.1, 19.4 and 19.2% in the summer, the spring, the fall and the winter, respectively.

Tea chemical composition was also varied during the processing (Hamid, 2006). The chemical composition in green tea was similar to the fresh tea leaves, while some compounds were oxidized to new compounds to become black tea (fermented tea) (Zhen, 2002). In oolong tea, fresh leaves are subjected to a partial fermentation stage before drying. Consequently, green and black teas differ noticeably in appearance, taste and chemical composition. Characteristic of oolong tea are between black and green tea. Table 2.5 shows that green tea had significantly higher catechins than that of black tea (Shukla, 2007). Catechins are the active compounds associated to the tea health benefit, therefore green tea has more benefit to health than others.

Table 2.5 Chemical composition of green and black tea (% w/w)

Components	**Green tea**	**Black tea**
Catechins	30-42	3-10
Flavanols	5-10	6-8
Other flavonoids	2-4	-
Theagallin	2-3	-
Gallic acid	0.5	-
Quinic acid	2.0	-
Theanine	4-6	-
Methylxanthines	7-9	8-11
Theaflavins	-	3-6
Thearubigins	-	12-18

Source : Shukla, 2007

Various extraction methods have been performed with many kinds of solvent in order to get higher yields of catechins (Uzunalic et al., 2006; Druzynska, 2007; Lee et al., 2008). Uzunalic et al. (2006) reported that water extraction provided high yields of catechins (448 g/kg dry mass of tea) when the extraction was performed at 80 °C for 20 min and 95 °C for 10 min. Druzynska (2007) was reported that six-fold lower catechins content was observed when the tea was extracted at room temperature for 15 min (72.8 g/kg dry mass of tea). While, Lee et al. (2008) reported that extraction at 60 °C for 60 min provided 9 g catechins content/ kg dry mass of tea. Due to the efficiency of water extraction, 95 °C and 10 min are used as the temperature and time of extraction in this experiment, respectively.

2.5 Tea Health Benefits

The Health benefits of tea have been intensively investigated. Epidemiological studies in the last decade indicated a role of tea in health and disease prevention, including antioxidative, antiarteriosclerotic, anticarcinogenic, antimutagenic, apoptotic, antidiabetic, antibacterial, and antiviral effects (Serafini et al., 1996; Kuroda and Hara, 2004; Muto et al., 2001; Steele et al., 2004; Yang and Koo, 2000; Hu et al., 2001).

Tea polyphenols posses antioxidant effects by preventing DNA damage, lipid hydroperoxide formation, and photograph-enhanced lipid peroxidation (Higdon and Frei, 2003). Tea polyphenols also exhibited scavenging activity against free radicals, superoxide radicals and peroxynitrite (Chen and Ho, 1995; Rice-Evans et al., 1997). As antimicrobial agents, tea leads to a reduction of enterobacteria, which produces ammonia, skatole and other harmful amines (Yamamoto et al., 1997). These can enhance the digestive protection systems in the intestines. Tea can inhibit the growth of *Vibrio parahaemolyticus* and *Staphylococcus aureus,* which are responsible for vibrio cholera and diarrhoeal diseases. It also reverses the methicillin-resistance in staphylococci hence enhancing the human immune system (Toda et al., 1989).

In terms of anti-inflammatory agents, tea catechins and quercetin inhibit the relief of lysosomal enzymes, the chemiluminescence response and the production of free radicals associated with neutrophil functions related to the preventing of heart disease and strokes (Tijburg et al., 1997). In addition, tea theaflavins have been reported to inhibit DNA single strand

breakdown and scavenged electrophilic free radicals to prevent oxidation and mutagenesis processes in the body (Shiraki et al., 1994).

A protective effect on rectal cancer incidence was also observed in Chinese female drinking tea regularly in Hebei province (Zhang et al., 2002). A study in Japan illustrated that there was a delay in cancer occurrence in the people regularly consuming green tea (Imai et al., 1997). The incidence of colorectal cancer was found to be lower in patients who had consumed over ten cups of green tea per day (Nakachi et al., 2000). Tea have been reported to suppress cell growth and induced apoptosis through mitochondrial depolarization, activation of caspase-3 and cleavage of DNA fragmentation factor-45 in human endothelial ECV 304 cells (Jiang et al., 1996). The induction of apoptosis by tea was also indicated by cleaving and condensing nuclear chromatin and DNA hypoploidy (Yoo et al., 2002). The authors suggested that tea exerted at least part of its anticancer effect by inhibiting angiogenesis through inducing endothelial apoptosis.

Tea has antidiabetes activity, indicated by its ability to suppress glucose transporters in the intestinal epithelium resulting in the reduction of dietary glucose intake (Kobayashi, 2000). A reduction in oxidative damage of lymphocyte DNA had been observed in diabetic patients uptaking quercetin and tea (Lean et al., 1999). Tea also had beneficial effects against viral infection (Pillai et al., 1999). Tea polyphenols apparently inhibited rotavirus propagation in monkey cell cultures and influenza virus in animal cell cultures (Yamamoto et al., 1997). The authors also claimed that several polyphenols inhibited retrovirus for human immunodeficiency virus (HIV) propagation by inhibiting reverse transcriptase, an enzyme allowing the establishment of the virus in host cells and then breaking the virus life cycle.

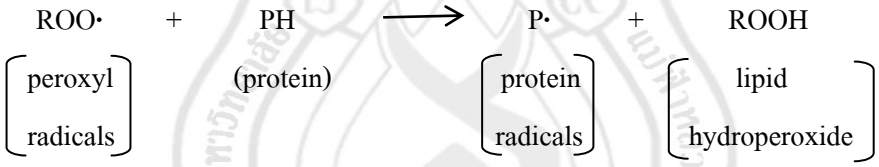
As a result of the enormous health benefits of tea, tea consumption has become more popular. As mentioned above, tea total polyphenol mainly contributed to those health benefits of tea with spesific modes of actions as described in the following section.

2.6 Tea Antioxidant Mode of Action

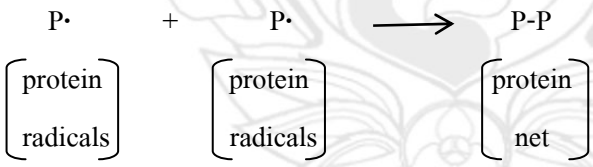
Oxidative reactions of lipids and proteins are a major cause of chemical deterioration in food. Free radical oxidation of lipids and proteins derives from reactive oxygen species (ROS) are generated during food processing and storage (Stadtman et al., 2003). Free radical species can

react directly with the protein or other molecules, such as lipids and carbohydrates, forming products that subsequently react with the protein (Salminen, 2009).

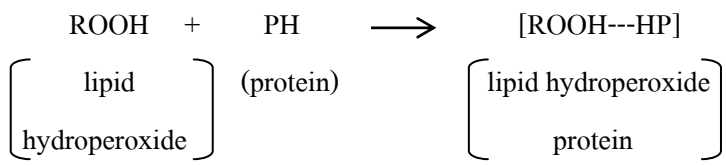
Primary lipid oxidation products (hydroperoxides) and secondary lipid oxidation products (aldehydes and ketones) can react with proteins, and cause protein oxidation (Viljanen, 2005). There are three reactions involved in protein oxidation. Firstly, protein oxidation occurs via free-radical reactions in which peroxyl radicals (ROO·) formed during lipid oxidation can abstract hydrogen atoms from protein (PH) as shown in Eq. 2.1. Secondly, the protein radicals (P·) create a protein net (P-P) due to cross-lingking (Eq. 2.2). Thirdly, the protein oxidation process can occur via non-covalent complex formation by both electrostatic and hydrophobic attractions (Eq. 2.3). Electrostatic and hydrophobic attraction can occur from the reaction of lipid hydroperoxide (ROOH) or secondary lipid oxidation products and the nitrogen or sulfur centers of reactive amino acid residues of the protein (PH). The secondary lipid oxidation products are mainly aldehydes and ketones, breakdown products of lipid hydroperoxides (Viljanen, 2005).

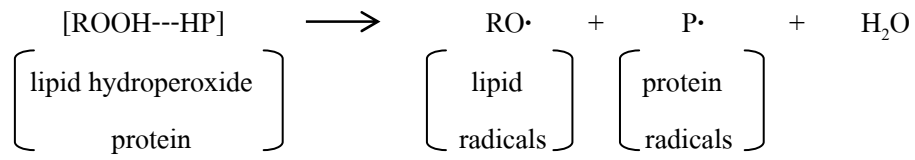


(2.1)



(2.2)





(2.3)

Figure 2.7 shows the possible protein oxidation pathways in the presence of oxygen and lipid oxidation products. Firstly, hydrogen atoms from protein (PH) react with a non protein radical (A•) and generates a protein radical (P•) (Eq. 2.4). There are three possible ways for protein radical reactions, including free radical transfer (Eq. A), oxidation (Eq. B) and cross-lingking (Eq. C). In the free radical transfer reaction, protein radicals (P•) catch hydrogen from other compounds to form non radical proteins or protein hydroperoxide. In the oxidation reaction, protein radicals (P•) is directly reacting with oxygen leading to protein peroxyl (POO•) and finally scission. In the cross-lingking mechanism, there are three ways protein to oxidize, including cross-lingking with lipid oxidation products such as hydroperoxides or aldehydes, interacting two protein radicals to form proteins net (P-P) and reacting protein peroxyl radicals with protein to form oxygen lingked protein dimers.

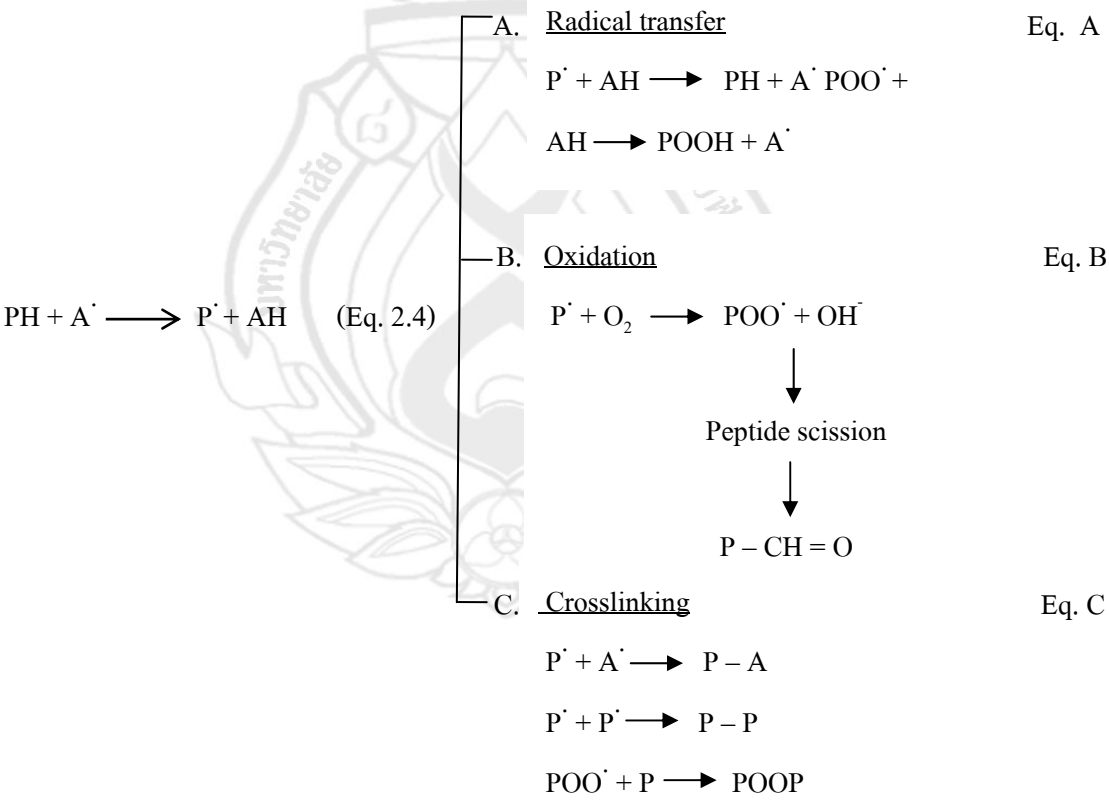


Figure 2.7 Protein oxidation pathways via A) free radical transfer, B) oxidation and C) cross-lingking; PH = protein, P• = protein radical, AH = any molecule with abstractable hydrogens, A• = non-protein radical, POO• = protein peroxyl radical, POOH = protein hydroperoxide

Source : Salminen (2009)

Interactions between lipids and proteins have a significant effect on the progress of oxidative reactions in foods. Oxidation reactions also have an impact on the charge and conformation of the protein three-dimensional structure (exposure of hydrophobic groups, changes in secondary structure and disulfide groups), loss of enzyme activity, and the nutritive value loss (loss of essential amino acids) (Viljanen, 2005). Oxidation in the human body has been linked to the changes occurring while aging, and particularly in a variety of diseases and disorders, such as infectious diseases, autoimmune diseases as well as neuropsychiatric and neurological disorders (Levine, 2002).

There are three mechanisms of tea polyphenols antioxidative reaction, including (1) scavenging reactive oxygen species, (2) chelating transition metal ions and (3) modulating oxidant/antioxidant enzymes or genes (Ho et al., 2009). Tea catechins have been shown to scavenge reactive oxygen species which may play important roles in carcinogenesis. The scavenging mechanism of tea catechins in DPPH (2,2-diphenyl-1-picrylhydrazyl) oxidant system are shown in Figure 2.8 and 2.9. As shown in Figure 2.8, an initial one electron oxidation of catechin on the B-ring by a DPPH radical generates a catechin (or epicatechin) phenoxyl radical. This phenoxyl radical can be tautomerized to the corresponding quinone B-ring to form compounds 3 and 4 or 5 and 6 (Figure 2.8). However, the antioxidant mechanism of EGC and EGCG were different from that of C (or EC) in the DPPH oxidant system. As shown in Figure 2.9, one electron attacked DPPH radical, generating EGC phenoxyl radical and tautomerized to *o*-quinone. This quinone attacks the C-2' of another EGC (or its ester) to form theasinensin A or C (Ho et al., 2009).

The effects of tea polyphenols may also result from the chelation of metal ions. C can chelate iron to form a stable complex as shown in Figure 2.10 (Liao et al., 2001). Tea manifests chelating activity *in vivo*, as indicated by the lower absorption of dietary iron and by the decreased body iron balance in the people who regularly consume tea (Wilson and Temple, 2004). This chelating ability is important because it protects iron-loaded hepatocytes from lipid peroxidation by removing iron from these cells (Liao et al., 2001). Tea catechins chelate copper ions and this mechanisms has also been suggested to protect low-density lipoprotein (LDL) from peroxidation (Yokozawa et al., 1997). Catechins may also effect signal transduction pathways, modulate many endocrine system, and alter hormones and other physiologic processes as a result of their binding of these metals/enzyme cofactors (Kao et al., 2000).

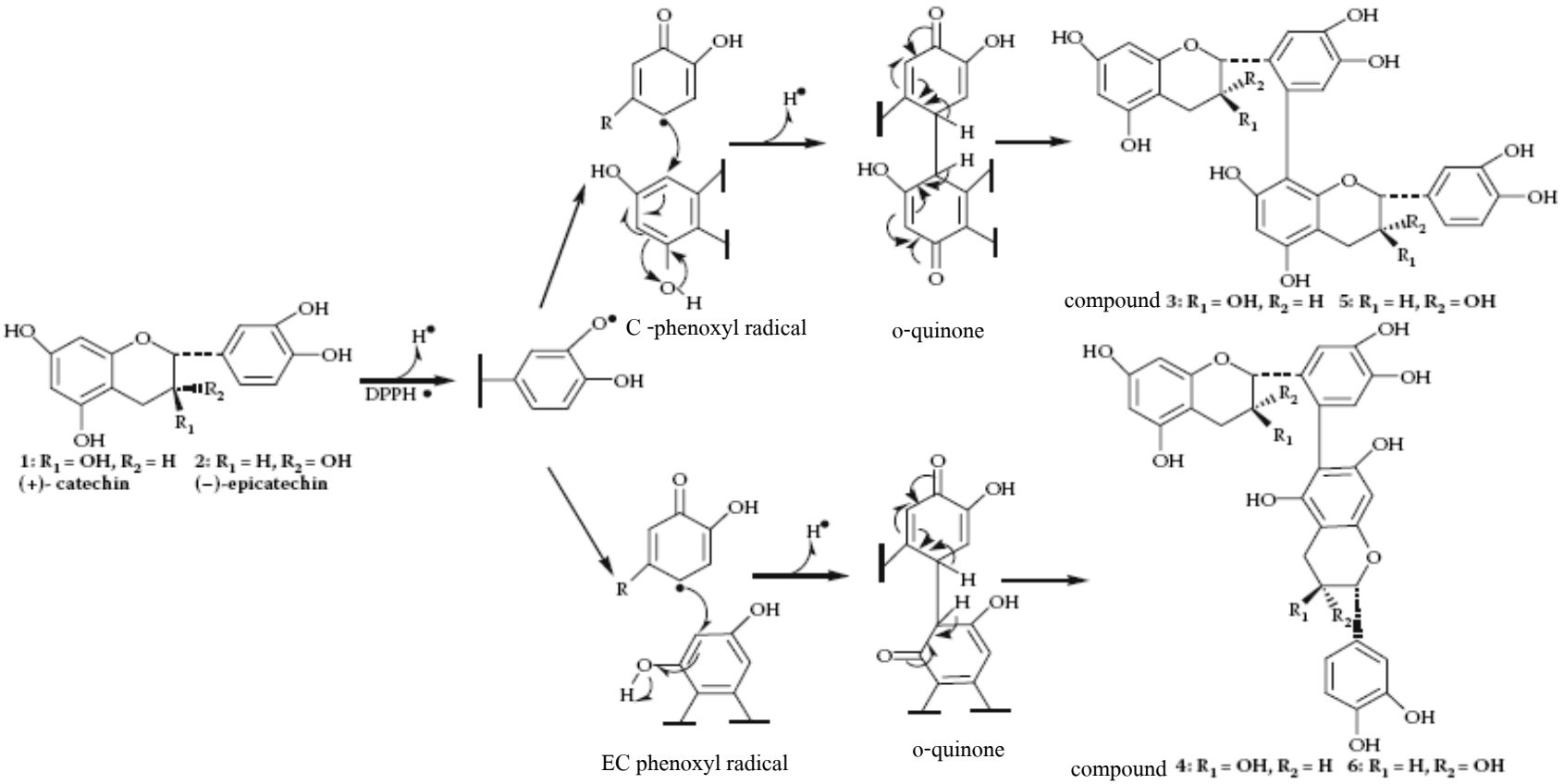


Figure 2.8 Scavenging mechanism of C and EC

Source : Ho et al. (2009)

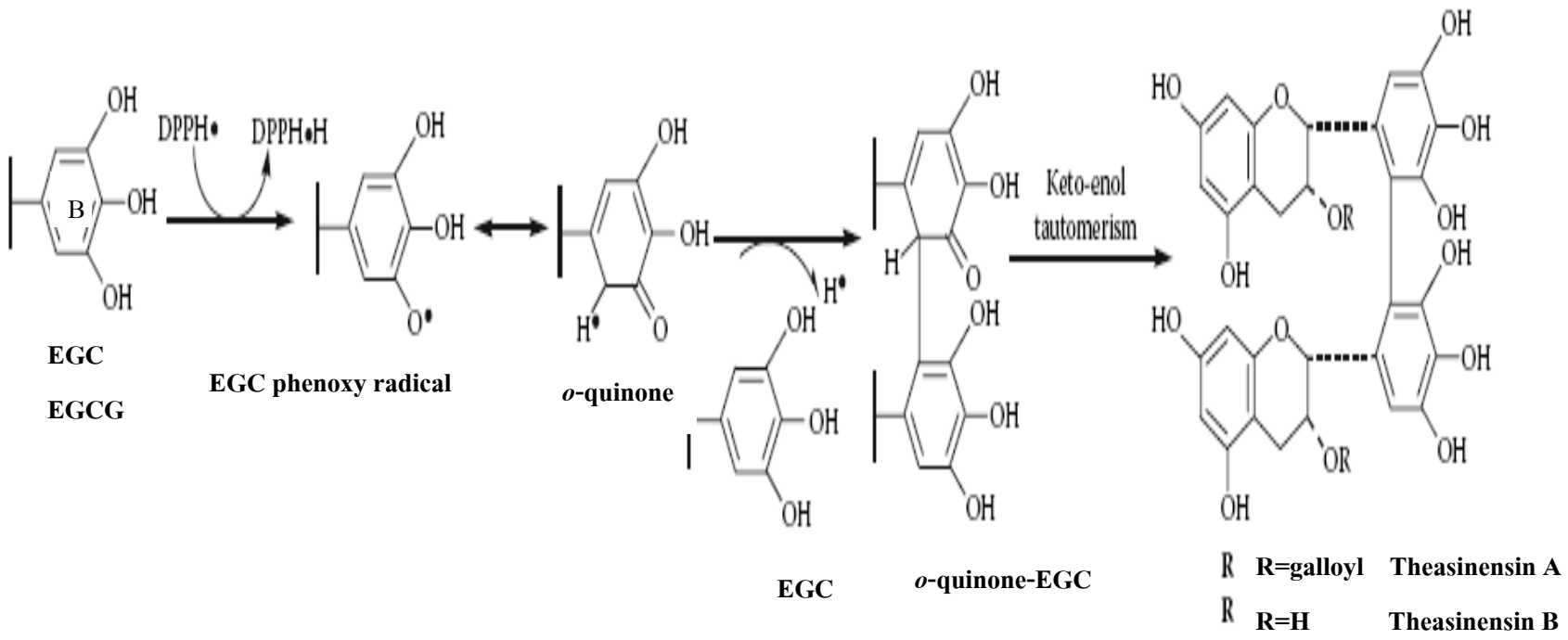


Figure 2.9 Scavenging mechanism of EGC and EGCG

Source : Ho et al. (2009)

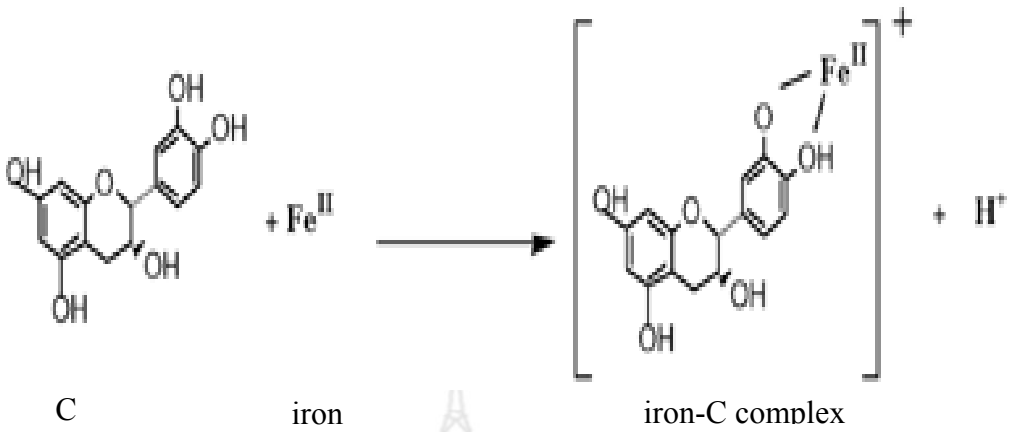


Figure 2.10 Chelating mechanism of catechin

Source : Bodini et al. (2001)

Tea polyphenols perform their antioxidant activity through regulating enzymes or genes related to oxidation/antioxidation. Tea polyphenols enhance the expression of intracellular endogenous antioxidants such as glutathione, glutathione reductase, glutathione peroxidase, glutathione-S-reductase, catalase, and quinine reductase (Ho et al., 2009). Polyphenols also stimulate antioxidant transcription and detoxification defense systems through antioxidant response elemen (ARE) with the coordination of endogenous antioxidants involving glutathione and its related enzymes to execute their antioxidative abilities in biological systems. Lin et al. (1998) found that catechins influenced free radical generation through the reduction of NADPH–cytochrome P-450 reductase activity. Tea polyphenols also inhibit the activity of cyclooxygenase (COX-2 and 5-, 12-, and 15-lipoxygenase), enzymes participating in enzymatic lipid peroxidation in human colon mucosa and colon tumor tissues (Ho et al., 2009). Studies on RAW 264.7 mice macrophages revealed that theaflavins, in particular Theaflavin (TF)-3,3′-Digallate (DG), effectively inhibit the activation of transcription nuclear factor ΚB (NFΚB), preventing the expression of an inducible nitric oxide synthase (iNOS) gene in mRNA and, as a consequence, contributes to a decrease in the synthesis of inducible nitric oxide synthase to prevent nitric oxide (NO) generation (Ho et al., 2009).

2.7 Tea Antimicrobial Mode of Action

Many researchers have investigated about antimicrobial activity of tea polyphenols against various strains of bacteria and their main components (Sakanaka et al., 2000; Wang et al., 2000; Yoda et al., 2004). Sakanaka et al. (2000) reported that tea polyphenols, especially EGCG, ECG and GCG, at the concentration of 0.25-1,00 mg/ml can inhibit the growth of *Pseudomonas gingivalis*. Tea polyphenols at a concentration of 0.25 mg/ml also showed significant inhibitory effects against carcinogenic bacteria such as *Streptococci, Streptococcus mutans* and *Streptococcus sobrinus* (Wang et al., 2000). Yoda et al. (2004) reported that EGCG at 50–100 μg/ml can inhibit the growth of *Staphylococcus* strains and *Helicobacter pylori* and at 800 μg/ml of EGCG was required to inhibit gram-negative rods.

The antibacterial activity of tea catechins showed that polyphenols may change the function of the cytoplasmic membrane, which selectively controls the permeability between the internal and the external cell (Miller, 1995; Yanagawa et al., 2003). Moreover, the authors claimed that the formed phenolic-protein complex in the cytoplasmic membrane may increase the susceptibility of microorganisms. Catechins activities may increase with the inactivation of enzymes in the cytoplasmic membrane, result in leakage or autolysis (Neu and Gootz, 2005) (Figure 2.11). Gram-positive bacterial cell walls contain thick peptidoglycan and the bacterium may or may not be surrounded by a protein or polysaccharide envelope, whereas gram-negative bacterial cell wall contains thin peptidoglycan, lipopolysaccharide, lipoprotein, phospholipid and protein. The peptidoglycan layer is essential for the survival of bacteria in hypotonic environments (Neu and Gootz, 2005). The loss or damage of these layers destroy the bacterial cell walls permeability, resulting in microorganism death. In addition, the lipopolysacharide in the cell membrane of gram negative bacteria provides a barrier to many antimicrobial agents, rendering these bacteria more resistant to certain agents than gram positive bacteria (Miller, 1995; Yanagawa et al., 2003; Ahn et al., 2004).

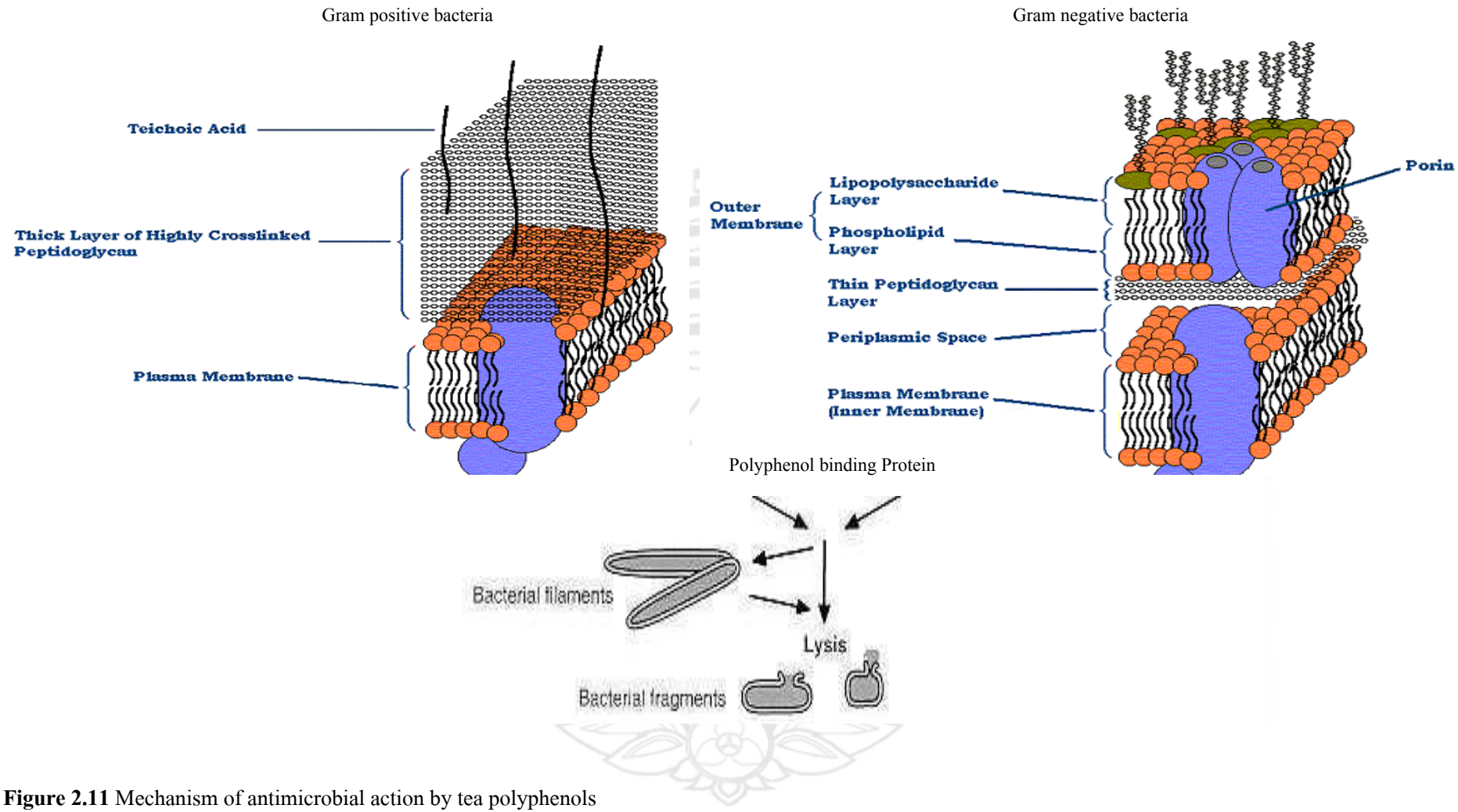


Figure 2.11 Mechanism of antimicrobial action by tea polyphenols

Source : Yanagawa, 2003; Neu and Gootz, 2005

2.8 Microbials Associated with Foods

Microorganisms are associated with foods both as beneficial agents and as major causes of spoilage and economic loss. Microbial spoilage is the major cause of food deterioration. Current losses to the food industry are caused by microbial spoilage are estimated at several million pounds annually in the UK (Spencer, 2001). There are many microorganisms that cause food spoilage, but *Escherichia coli, Listeria monocytogenes, Salmonella typhimurium* and *Staphylococcus aureus* are mostly found in foods (USDA, 2006). Therefore, this section will only focus on those pathogenic microorganisms.

2.8.1 *Eschericia coli*

Eschericia coli, a member of the Enterobacteriaceae family, is gram negative, motile, nonsporulating, and rod is shaped facultative anaerobic bacterium. It lives naturally in the intestine of warm-blooded animals. It can grow at 10-50 °C with the optimum growth temperature of 37 °C and the minimum pH for growth is 4-4.5. *E coli* can be classified into four groups, including enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (EHEC) and enteroinvasive *E. coli* (EIEC). EPEC generally causes diarrhea when subjected to 10^5 to 10^8 cells after 12-36 hrs of ingesting. It also interups the function of nutrient absorption and secretion in the small intestine (Ray, 2001). ETEC causes illness after ingestion of 10^8 to 10^{10} cells per 12-36 hrs of contamination and produces heat stabile and labile toxins (Bell and Ayriakides, 2002). EHEC produces verotoxin causing haemorrhagic colitis, haemolytic uraemic syndrome and thrombotic thrombocytopaenic purpura (Natato and Kaper, 1998). Ineffective doses of EIEC is about 10^6 cells, and causes invasive bacillary dysentery, ulceration, fever, inflammation, abdominal pains, malaise and diarrhea (Adam and Moss, 2000).

E. coli is transmitted to humans primarily through consumption of contaminated foods, such as raw or undercooked ground meat products and raw milk. Faecal contamination of water and other foods, as well as cross-contamination during food preparation, also lead to infection. Outbreaks of *E. coli* also occur in undercooked hamburgers, dried cured salami, unpasteurized fresh-pressed apple cider, yogurt, cheese and milk. An increasing number of outbreaks are associated with the consumption of fruits and vegetables (sprouts, lettuce, coleslaw,

salad) whereby contamination may be due to contact with faeces from domestic or wild animals at some stage during cultivation or handling (Mosqueda-Melgar *et al.,* 2007).

2.8.2 *Salmonella typhimurium*

Salmonella, a member of the Entereobacteriaceae family, is a gram negative, rod shaped, facultatively anaerobic, and non spore forming. It ferments glucose and mannose with the production of gas and acid (Kim, 2003). It is oxidase-negative, catalase positive, and generally produces hydrogen sulfide. It can grow at a wide temperature range, with the optimal temperature of 37 °C and the minimum growth temperature of 2-4 °C. *Salmonella* can grow at the pH ranging from 4.5-9.5, with the optimum pH of 6.5-7.5 (Kim, 2003). *S. typhimurium* is the most frequent serotypes responsible for human illness (CDC, 2001).

Salmonella is found naturally in the gastrointestinal tract of domesticated and wild animals, birds, pets and insects (Ray, 2001). Unpasteurized milk, raw eggs, meat and unprocessed food or animal by-products are also the sources of *Salmonella* (CDC, 1984; Khurana and Kumar, 1993; Kim, 2003; Ahn, 2004). Data from many of the industrialized nations suggest that *salmonella* is the most important cause of foodborne disease (Salmonellosis). It causes illness at low doses (as few as 10 bacteria cells per gram food) (Kim, 2003). The incidence of Salmonellosis is reported to have increased in recent years. The USDA (2006) reported that 696,000-3,840,000 cases of foodborne illness are due to *S. typhimurium* infection with 870-1,920 deaths annually.

2.8.3 *Listeria monocytogenes*

Listeria monocytogenes is a gram positive, regular, short-rod, facultative anaerobic, catalase positive, oxidase negative, non-sporulating rod and motile (Kim, 2003). It is generally distributed in nature and is isolated from soil, vegetation, water, intestinal tract, milk and raw meat products. The optimum temperature range is from 0-42 °C. *L. monocytogenes* can survive at low temperatures, low water activity and in high salt. However, it is sensitive to high temperature (> 62.8 °C).

Listeriosis, an illness caused by *L. monocytogenes* is an opportunistic disease, which depends on an individual's health infection. It generally appears as a mild disease in healthy people, while it causes very serious disease to fetuses, unborns, infants, pregnant women and immuno-deficient people. The symptoms include fever, fatigue, nausea, vomiting and diarrhea.

Several listeriosis causes meningitis and septicemia, which occasionally result in death (Kim, 2003).

2.8.4 *Staphylococcus aureus*

Staphylococcus aureus, a member of the Micrococcaceae family, is a gram positive, facultative anaerobic bacterium. It is a spherical coccus that appears in pairs with short chains or bunched and grape-like clusters. *S. aureus* is commonly found in the nose, throat, hair, and skin of more than 50 percent of healthy individuals (Fung, 1999). Infected wounds, sneezing, and coughing by individuals with respiratory infections may also be sources that transmit the bacteria. *S. aureus* can survive for extended periods in a dry environment, including in foods with water activities of less than 0.86. It also can survive in a medium with 3.5 M sodium chloride. The growth temperature ranges from 15-45 °C.

S. aureus ranks as one of the most prevalent causes of gastroenteritis worldwide. The toxin level is reached when the population exceeds 5 log CFU/g (Jablonski and Bohach, 1997). The symptoms are nausea, vomiting, retching, abdominal cramping and prostration for about four to six hours after consumption (Kim, 2003). In several cases, the others symptoms, including headaches, muscle cramping and transient changes in blood pressure and pulse rate can also occur, which generally takes about 24 to 72 hrs to recover.

Toxins caused by *S. aureus* are formed in food. It will not be destroyed by boiling due to their heat stability (Kim, 2003). Foods that require considerable handling during preparation and that are kept at slightly elevated temperature after preparation are frequently involved in *S. aureus* poisoning. Foods associated with *S. aureus* include meat and meat products, poultry and egg products, salads (egg, tuna, chicken, potato and macaroni), bakery product (cream-filled pastries, cream pies and chocolate), sandwich fillings, milk and dairy products. Intoxication *S. aureus* occurs in humans because the food has not been kept hot enough (60°F to 140°F, or above) or cold enough (7.2°F to 45°F, or below). The highest incidence generally occurs in the summer, therefore it is essential to keep cooked food refrigerated or hot food at higher temperatures (Fung, 1999).

2.9 Fruit Juice Deterioration

Fruit juice has high nutrition, vitamin and mineral content as shown in Table 2.6 (Ashurst, 2005; Berryman, 2007). Fruits can be contaminated with pathogenic and spoilage microorganisms either during their growing in fields, orchards, vineyards or greenhouses, or during harvesting, post-harvest handling, distribution and processing (Cherry, 1999; Beuchat, 2002). Fresh fruit have a natural protective barrier that acts effectively against most plant spoilage and pathogenic microorganisms. However, this protection is eliminated during processing, consequently exposing the fruit flesh to unfavorable environmental conditions as well as to a possible contamination with pathogenic microorganisms including bacteria (Brackett, 1996). Hence, the number of documented outbreaks of human infection associated with the consumption of fruit juices were reported as shown in Table 2.7. The Food and Drug Administration (FDA) has established regulations for juice manufacturing that treat for commercial preparation, capable of reducing pathogenic load by a minimum of 5 log (US FDA, 2002)

Table 2.6 Nutrition composition of fruit juice

Nutrition	Apple Juice	Orange Juice	Melon Juice	Watermelon Juice
Calories (g)	47	42	34	30
Protein (g)	0.06	0.59	0.84	0.61
Fat total (g)	0.11	0.14	0.19	0.15
Carbohydrates (g)	11.68	6.85	8.16	7.55
Fiber total (g)	0.10	0.20	0.90	0.40
Sugar total (g)	10.90	8.40	7.86	6.20
Calcium (mg)	7.00	8.00	9.00	7.00
Iron (mg)	0.37	0.44	0.21	0.24
Potassium (mg)	119.00	175.00	267.00	112
Vitamin C (mg)	0.90	34.4	36.70	8.10
Vitamin A (IU)	1.00	175.00	3382.00	569.00
Vitamin E (mg)	0.03	0.20	0.05	0.05
Vitamin K (mcg)	0.00	0.10	2.50	0.10

Source : Arshurst, 2005; Berryman, 2007

Table 2.7 Reported outbreaks of fruit juice

Year	Juice	Microorganism	Victim
1995	Orange	*Salmonella enteretidis*	60 visitors affected (Florida
		Eschericia coli O157:H7	theme park, USA)
1996	Apple	*Listeria monocytogenes*	48 cases (Washington DC)
2000	Citrus	*Salmonella enteretidis*	14 cases (Multistate, US)
2006	Watermelon	*Salmonella typhimurium*	7 cases (California, US)
2006	Strawberries,	*Eschericia coli* O157:H7	7 cases (Manhattan, US)
	Bluberries		

Source : Center of Disease Control and Prevention, 2007

A number of preservation methods are applied in the fruit juice industry design to extend the shelf life of the food products, including heat and food additives. These pathogenic microorganism can be easily eliminated through heat, but quality losses such as sensorial and nutritional attributes extensively occur (Martinez and Belloso, 2005). Nevertheless, significant efforts are leading to the development of using natural antimicrobial (Corte et al., 2004; Kiskot and Roller, 2005), due to the high demand of healthy, fresh like and safe foods as consequence of increased assurances of safety and quality.

Different studies have been carried out about the effects of organic acids directly or indirectly adding to fruit juices over pathogenic spoilage microorganism. Uljas and Ingham, (1999) reported reductions of ≥ 5 log CFU/ml of *E. coli* 0157:H7 and *S. typhimurium* DT104 in unpasteurized apple stored at 35 °C for 6 hrs. However, when a combination of sorbic acid with freezing-thawing treatment were applied, a 5 log CFU/ml decrease of *E. coli* O157:H7 was achieved shorter time (4 hrs). Ceylan (2003) observed that Cinammon (0.1-0.3% w/w) effectively reduced 2 log CFU/ml of *E. coli* at 25 °C after 3 days and its combination with 0.1% sodium benzoate or potassium sorbate synergistically eliminated 5.3 log CFU/ml of this bacteria. The effect of the different temperature used to store the juices also has been reported by several researchers (Yuste and Fung, 2002; Raybaudi-Massilia et al., 2008).

The preservative action of antimicrobials depends on the type, genus, species, and strain of the tested microorganisms. The efficiency of an antimicrobial agent also depends to a great extent on environmental factors, such as pH, water activity, temperature, atmosphere, initial microbial load, acidity and food matrix (Davidson, 2001). Among those factors, pH is the most influential factor that enhances the antimicrobial properties of the antimicrobial agent (Davidson, 2001). Therefore, it is important to know the specific characteristics of the food system that needs to be preserved. It is important because a high proportion of lipids could limit the antimicrobial effectiveness of some antimicrobial agents, which is due to the linkage between the food lipids and the antimicrobial substances with hydrophobic characteristics.

Watermelon juice is a beverage with high nutrition, minerals and vitamin content (Ashurst, 2005). This products is regarded as a potentially hazardous food by the Food and Drug Administration (FDA) (FDA, 2001) because the juice may favor the growth of pathogenic microorganisms due to their low acidity (pH 5.2 to 6.7). Outbreaks of *Salmonella spp*. and *E. coli* have been linked with the consumption of fresh-cut as well as the juice of watermelon (CDC, 1991; Mohle-Boetani et al., 1999; Powell and Leudtke, 2000; CDC, 2001; Meng et al., 2001; FDA, 2001; CDC, 2002). Consumers demand for greater assurances of safety, but also greater maintenance of sensorial characteristics in food. These demands lead researchers to intensively seek for natural occuring microbials that will provide prolonged freshness without loss to nutritional value.

Tea as a natural preservative has been reported to posses antimicrobial activity against some pathogenic bacteria (Masashi et al., 2000; Amarowicz, 2004; Bong-Jeun et al., 2003). However, the effectiveness of natural preservatives in fruit juice depends on juice composition, such as pH, water activity, redox potential and nutrients (Fortuny and Belloso, 2003). In this experiment, watermelon juice has been used as a food model system. This is due to its availability to provide a greater medium for bacteria growth compared to those of other kinds of fruit juice with higher acidity.

2.10 Meat Deterioration

Meat is one of the very rich nutrition foods containing high protein and important micronutrients. Meat also has a lot of fats as shown in Table 2.8 (USDA, 2005; Williams, 2007). These make meat and meat products very susceptible to microbial contamination and lipid oxidation, which leads to a sensorial unacceptable and unsafe for the consumer (Kanatt et al., 2008). As shown in Table 2.9, some common pathogens found in meat and meat products are *Staphylococcus aureus, Listeria monocytogenes, Salmonella typhimurium* and *Eschericia coli.*

Table 2.8 Nutrition composition of meat

Nutrition	Beef	Chicken	Pork
Energy (KJ)	527	564	504
Protein (g)	22.7	19.3	21.3
Total Fat (g)	3.8	6.4	3.9
Total Omega 3 (g)	0.11	0.06	0.04
Iron (mg)	2.0	0.9	1.0
Zinc (mg)	4.2	1.6	2.1
Riboflavin (mg)	0.15	0.13	0.2
Vitamins B12 (mcg)	1.1	0.4	0.7

Source : USDA, 2005; Williams, 2007

Table 2.9 Microorganisms associated with illnesses in meat and meat products

Meat and meat products	Microorganisms
Beef	*L. monocytogenes, Campylobacter jejuni*
	E. coli, S. typhimurium, S. aureus
Chicken	*S. typhimurium*
Beef meatball	*E. coli, S. typhimurium*
Sausage	*E. coli, S. typhimurium*
Pork	*S. typhimurium, S.aureus, Clostridium perferingens*

Source : Hubbert et al., 1996

Microbial deterioration in meat depends on environmental conditions, such as sterling temperatures, relative humidity and the pH of the product as well as the inherent characteristic of the meat itself. Microbial contamination to meat and meat products leads to some changes, including acid production, gas production, slims formation, mold growth, bacterial greening, the formation of green rings or the development of green cores (Hubbert et al., 1996). These all make the meat and meat products unacceptable and harmful to consumer health. The International Commission on Microbiological Specifications for Foods has established regulations to assure consumer food safety that the microorganism do not exceed 100 CFU/g of food (Alves, 2006). Therefore, many types of meat preservatives are applied to the meat, especially the ready-to-eat meat in order to control the harmful microorganism, including drying, freezing and food additives (Odumero, 2006; Aymerich, 2007; Coma, 2008). However, there are some limitation of using those synthethic preservatives in the meat as a results of their potent health effects. As a result, various investigations have been performed on natural preservatives in meat and meat products (Kanatt et al., 2008; Solomakos et al., 2008). The major deterioration of meat product is shown in Table 2.10.

Table 2.10 The major deterioration of meat and meat products by microorganisms

Meat Product	Deteriorated attributed
Bacon	Chessy texture, souring, rancidity, discoloration, slime formation
Vacuum packaged (cured)	Cabbage odor taint
Ham	Surface slim, gassiness or puffiness, green discoloration
Sausage	Surface slim, gass production, greenish discoloration
Fermented sausage	Slime spots (disoloration)
Canned meats	Gas, putrefaction, souring, discoloration
Poultry	Off odor slim
Vinegar-pickled meat	Cloudy or ropy brine
Fresh red meat	Off odor, sliminess, discoloration, moldiness, whiskers, white spot, black spot, bone taint, gassiness, souring
Vacuum packaged (fresh)	Acidity, sweetness, rancidit

Source : Hubbert et al., 1996

Oxidation of lipids is also a major cause of deterioration in the quality of meat and meat products due to the high lipid content (Pearson et al., 1977). Oxidation of fats results in the replacement of an oxygen ion for a hydrogen ion in the fatty acid molecule. This substitution destabilizes the molecule and makes it possible for other odd chemical fragments to find a place along the chain. These reactions can lead to loss of nutritional value, quality change such as color, texture and flavor and the formation of organic free radicals (Hubbert et al., 1996). Oxidative stability of meats is related to the degree of saturation of the lipid fraction. Unsaturated fats are more susceptible to oxidation than are saturated fats (Wilson et al., 1976; Pearson et al., 1977).

Beef consumption is increasing as a result of its health beneifts (USDA, 2002). This demand has led to the development of a variety of beef products (Ahn, 2003). Beef as source of iron content can posses high oxidation susceptibility due to its catalzying ability in the Fenton and Haber-Weiss reaction (Ho et al., 2009). However, lipid oxidation and microbial contamination mainly causes quality deterioration in beef products (Levine et al., 2002). Due to increasing demand of beef products (Peter-Blecha and Schreder, 2009), beef safety and quality have received high priority over the last decade. To meet these needs, the appropriate preservation technologies must be applied. Beef is prone to both microbial, oxidative spoilage and discoloration and therefore, it is desirable to use a preservative (Nissen et al., 2004; Kanatt et al., 2008).

CHAPTER 3

ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF CHIANG RAI COMMERCIAL ASSAM GREEN TEA INFUSION *IN VITRO*

3.1 Introduction

Recently, tea (*Camellia sinensis*) is widely consumed as a result of its health benefits (Almajano et al., 2008). Most of the tea health benefits are regarded to be its antioxidant and antimicrobial properties. These result from tea rich phenolic compounds (Wang et al., 2000). However, tea compositions, particularly the active phenolic compounds, are affected by various parameters, such as the cultivation area, season and processing (Cheng-Chun et al., 1999; Zhen, 2002). Green tea (unfermented tea) has been reported to be more healthy compared to oolong (semi-fermented tea) and black tea (fermented tea) (Gramza and Korczak, 2005; Wang et al., 2000; Yilmaz, 2006; Almajano et al., 2008). Therefore, various green tea products are available as a health drink in the market all around the world.

In Thailand, Chiang Rai province is the biggest tea cultivation area, especially for assamica (*Camellia sinensis* var. *assamica*). From preliminary observations, the Taiwanese style tea production process was modified and applied for the tea production in this area based on the owner experience. Consequently, different tea products with different qualities are produced and distributed to the market. So far, there is no report on Chiang Rai assam green tea infusion quality, particularly about their antioxidant and antimicrobial properties. In addition, there is an increasing demand of the natural food additive as a result of health concerns. Therefore, this work focused on an investigation of the antioxidant and antimicrobial activities of Chiang Rai commercial assam green tea infusions.

3.2 Experimental Procedure

Five Commercial assam green teas used in this experiment were purchased from the market in Mueng district, Chiang Rai in the year 2008, represented by *A, B, C, D* and *E*. In the following sections, there are description of green tea infusion preparation, their antioxidant activities in terms of total polyphenol, 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging, and catechins content determination and finally their antimicrobial activities by minimum inhibitory concentration.

3.2.1 Preparation of Assam Green Tea Infusion

Assam green tea infusion was prepared as previously stated by Uzunalic et al., 2006. Briefly, the assam green teas were ground and sieved with ~500 μm pore size and kept in a sealed plastic bag until used. A ground sample (62.5 g) was extracted with 250 ml of boiling distilled water for 10 min. Then, the extract was filtered through a Whatman No. 4 filter paper. The filtrate was collected and the final volume of the filtrate was made up to 250 ml with distilled water in order to get the assam green tea infusion concentration of 250 mg/ml. Then, various volumes of the 250 mg/ml assam green tea infusion was throroughly mixed with different volumes of distilled water in order to get the desired concentrations (0, 25, 50, 75, 100, 125, 150, 175, 200, 225 and 250 mg/ml) as shown in Table 3.1. The assam green tea infusion was sterilized by passing through a membrane filter with the pore size of 0.45 μm.

3.2.2 Microorganisms Preparation

All tested microorganism (*Staphylococcus aureus*; TISTR 1466, *Salmonella typhimurium*; TISTR 292, *Escherichia coli*; TISTR 780) were purchased from Microbiological Resources Center, Thailand Institute of Scientific and Technological Research (TISTR), except *Listeria monocytogenes,* which was purchased from DMST culture collection, Department of Medical Sciences Thailand. To prepare the bacterial culture, bacteria was cultured in 100 ml of nutrient broth (NB) at 37 °C for 24 hrs. The microbial concentration of the obtained culture was determined and diluted with 0.1% peptone water in order to get a culture final concentration of approximately 7.0-8.0 log CFU/ml. The growth curves of all cultures are shown in Figure B.1-B.4.

Table 3.1 Assam green tea infusion preparation

Volume of stock 250 mg/ml tea infusion (ml)	Distilled water (ml)	Tea final concentration (mg/ml)
0	20	0
2	18	25
4	16	50
6	14	75
8	12	100
10	10	125
12	8	150
14	6	175
16	4	200
18	2	225
20	0	250

3.2.3 Total Polyphenol Content

The total polyphenol content (TPC) was determined by Folin-Ciocalteu method (ISO, 2005a). Briefly, 1 ml of the tea solution and 5 ml of 10% (v/v) Folin-Ciocalteu reagent were thoroughly mixed in a test tube. After 30 min, 4 ml of 0.708 M sodium carbonate was added to the test tube and then the mixture was allowed to stand at room temperature for 1 hrs. The optical density of the obtained solution was determined by spectrophotometer (Lamda 35, Perkin Elmer Life and Analytical Sciences, Inc, USA) at the wavelength of 765 nm and the total polyphenol content was expressed as gallic acid equivalent. The standard curve of TPC is shown in Figure C.1.

3.2.4 Antioxidant Activity

Antioxidant activity of assam green tea was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging method (Miliaskus, et al., 2004). Fifty microliters of tea solution were added to 1,950 µl of 60 µM DPPH solution and mixed thoroughly. The mixture was left at room temperature for 30 min and then the absorbance was read at 517 nm by UV-

Visible spectrophotometer (Lamda 35, Perkin Elmer Life and Analytical Sciences, Inc, USA). Antioxidant activity was expressed as % inhibition and trolox (μmol/100 g dry basis). The IC_{50} of DPPH radical of each sample was also calculated to evaluate the concentration (mg/ml) of assam green tea infusion required to inhibit 50% DPPH radical formation.

3.2.5 Catechins Determination

HPLC method (ISO, 2005b) was used to determine the catechins, including catechin (C), epicatechin (EC), gallocatechin (GC), catechin gallate (CG), epigallocatechin (EGC), gallocatechin gallate (GCG), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) as well as gallic acid (G) and caffeine (CF) in the assam green tea infusion. For this study, the following equipment was used : a HPLC system Agilent Technology 1100 Series, equipped with a quaternary pump, a degasser, a wavelength, an auto-sampler with a reverse phase C18 column and a photodiode array detector (DAD). The binary mobile phase consisted of 13% (v/v) of acetonitrile and 87% (v/v) of 0.05% trifluoroacetic acid. The flow rate was kept constant at 2 ml/min for a total run time of 12 min. One mililiter of tea infusion was filtered through a polytetrafluoroethylene (PTFE) filter and the filtrate was collected in a small amber vial. Subsequently, 10 μl of the filtrate was injected to the HPLC. The concentrations of investigated catechins were determined based on the standard chromatogram data shown in Figure C.3.1-C.3.10.

3.2.6 Antimicrobial Activity Determination

Antimicrobial activity of tea infusion was tested against 4 selected pathogens using the agar diffusion method (Fernandez et al., 2005). Briefly, 1 ml microorganisms suspension of approximately 7 log CFU/ml was mixed with tempered nutrient agar and poured into a sterile petri dish. Subsequently, 50 μl of each tea infusion concentration (25-250 mg/ml) was aseptically applied to 6 mm diameter sterile filter disc and placed on the nutrient agar containing microorganisms in the petri dish. The same amount of sterile distilled water was used as the control. The petri dishs were incubated at 37 °C for 24 hrs. The antimicrobial activity of the assam green tea infusion was evaluated by measuring the inhibition zone against the tested organisms. The inhibition activity was expressed as the diameter of the inhibition zone. The lowest concentration that the first inhibitory effect observed was then defined as the minimum inhibitory concentration (MIC).

3.3 Results and Discussion

3.3.1 Antioxidant Activity

Total polyphenol content (TPC) and DPPH scavenging activity in terms of inhibition percentage of the assam green tea infusions are shown in Figure 3.1 and 3.2, respectively. It was found that mean TPC of *A, B, C, D* and *E* were 23.50, 20.18, 17.39, 15.95 and 15.85%, respectively. The TPC of the assam green tea infusions is consistent to their DPPH scavenging inhibition activity. The results showed that *A* significantly exhibited the highest inhibition activity (90.10%) and followed by *B* (82.81%), *C* (77.60%), *D* (75.39%) and *E* (73.96%), respectively ($p \leq 0.05$).

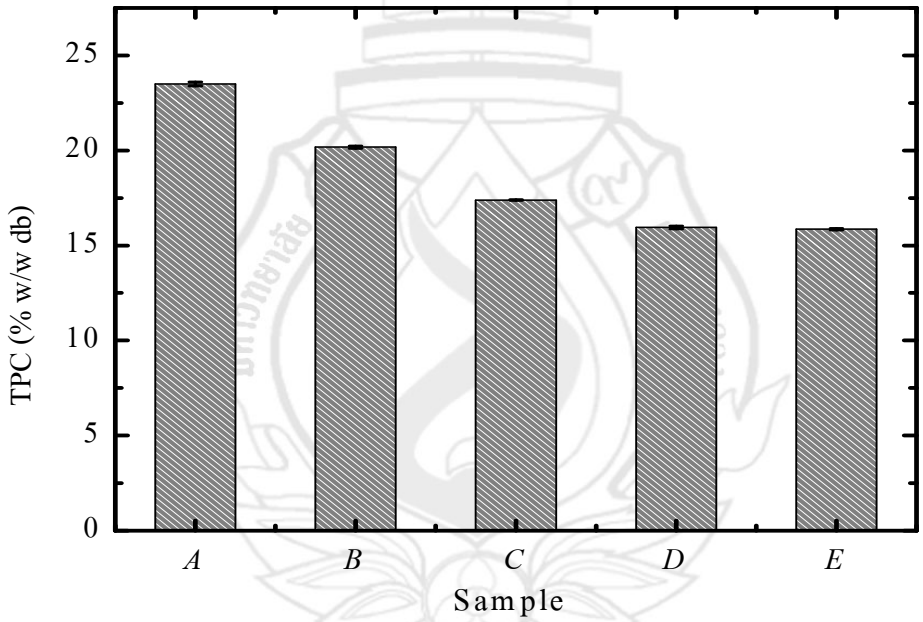


Figure 3.1 Total polyphenol content (% w/w dry basis) of assam green tea infusions

Table 3.2 shows antioxidant activity in terms of IC_{50} and trolox equivalent (TE) of the assam green tea infusion. The results showed that there was significant difference observed in IC_{50} for all assam green tea infusions ($p \leq 0.05$). *A* exerted the lowest IC_{50}, followed by *B, C, D* and *E,* respectively. Whereas, *A* was observed to have significantly higher TE (21,620 µmol trolox/100 g db) than *B* (19,390 µmol trolox/100 g db), *C* (19,860 µmol trolox/100 g db), *D* (18,310 µmol trolox/100 g db) and *E* (18,100 µmol trolox/100 g db), respectively. The results

showed that assam green tea infusion with high TPC exerted high antioxidant activity. These indicate that the antioxidant activity of assam green tea infusions were responsible by TPC. These results are consistent with Zhu et al. (2002), who reported that the strong DPPH scavenging activity of tea was contributed by the tea polyphenols.

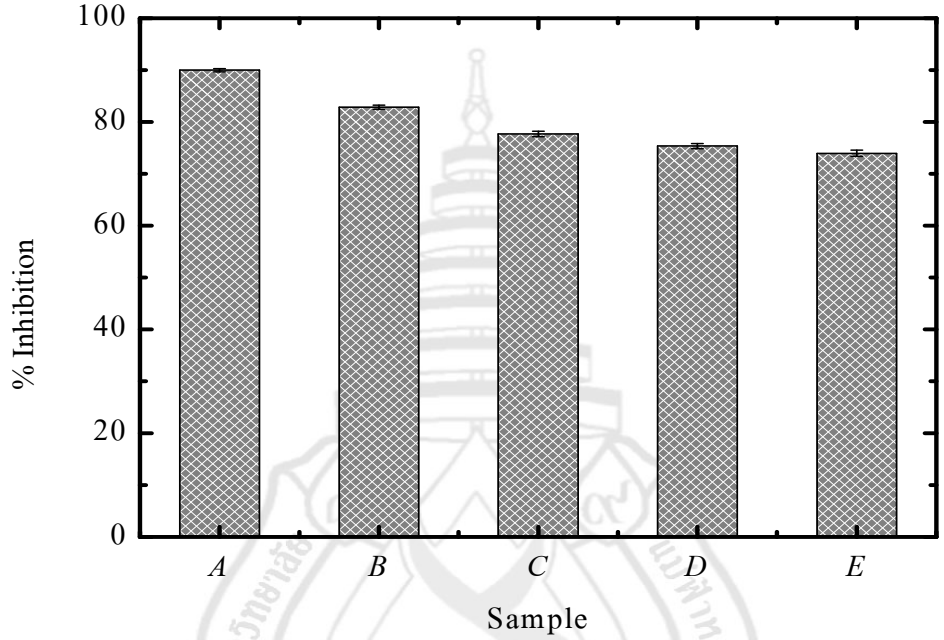


Figure 3.2 DPPH scavenging activity (% inhibition) of assam green tea infusions

Table 3.2 Inhibitory concentration (IC_{50}) and trolox equivalent (TE) values of assam green tea infusions

Sample	IC_{50} ± SD (mg/ml)	TE ± SD (μmol/100 g db)
A	25.23 ± 0.01^{a}	$21{,}620 \pm 1.77^{a}$
B	26.33 ± 0.05^{b}	$19{,}860 \pm 1.79^{b}$
C	27.43 ± 0.05^{c}	19,390 ± 3.08c
D	28.53 ± 0.08^{d}	$18{,}310 \pm 3.12^{d}$
E	29.63 ± 0.10^{e}	$18{,}100 \pm 3.68^{d}$

*a-d Means with different superscript letters within a column are signifantly different at $p \leq 0.05$

Table 3.3 shows some chemical composition in assam green tea infusions. It was observed that *A* has the highest EC (2.16% db), while *B* was found to have the highest EGC (3.37% db). It was also observed that *C* contained highest G (1.35% db), while it contained only 2.44% db EGC which was less than that of *A* (2.69% db). It was found that the average of catechins content for Chiang Rai assam green tea products are consistent within the same range of approximately 0.33, 1.64 and 2.62% db for C, EC and EGC, respectively. Whereas, these contents of US green teas were varied in wider ranges (Friedman et al., 2005). For instance, C content in the US green tea was in the range of 0.1 to 7.9% db and the EGC and EC content were varied from 0.1-14.2% and 0.1 to 6.3% db, respectively.

Table 3.3 Some chemical composition in assam green tea infusions

Samples	Catechins (% w/w dry basis)					
	CF	C	EC	EGC	G	EGCG
A	3.44 ± 0.46^{a}	0.54 ± 0.01a	2.16 ± 0.08^{a}	2.69 ± 0.45^{c}	1.21 ± 0.02^{b}	ND*
B	3.33 ± 0.46^{a}	0.37 ± 0.02^{b}	1.81 ± 0.19^{b}	3.37 ± 0.80^{a}	0.94 ± 0.06^{d}	ND*
C	2.89 ± 0.31^{b}	0.35 ± 0.05^{b}	1.59 ± 0.21^{c}	2.44 ± 0.59^{d}	1.35 ± 0.04^{a}	ND*
D	2.85 ± 0.15^{b}	0.38 ± 0.08^{b}	1.30 ± 0.52^{e}	2.89 ± 0.42^{b}	1.09 ± 0.15^{c}	ND*
E	2.31 ± 1.20^{c}	ND*	1.37 ± 0.80^{d}	1.34 ± 0.16^{e}	1.21 ± 0.15^{b}	ND*

a-e Means with different superscript letters within a column are significantly different at $p \leq 0.05$

*ND = non detectable

The results showed that *A* had the highest EC, followed by *B, C, E* and *D,* respectively. *B* had the highest EGC, followed by *D, A, C* and *E,* respectively. However *D* had the lowest EC compared to other infusions. *A* and *B* exhibited the higher TPC and antioxidant activity, whereas *D* had the lower TPC and antioxidant activity. These indicate that the high TPC and antioxidant activity of *A* and *B* were not only resulted from EC or EGC alone, but also resulted from a combination of those compounds (Gramza and Korczak, 2005; Almajano et al., 2008). Catechins acted as hydrogen ion donors to the radical species, thus terminating the chain reaction by converting the free radical to more stable products.

3.3.2 Antimicrobial Activity

Figure 3.3 to 3.12 show the antimicrobial activity of the assam green tea infusions against 4 bacterial strains, including *S. aureus, L. monocytogenes, E. coli* and *S. typhimurium.* In Figure 3.3, it was found that all commercial assam green tea infusion at the lowest concentration of 25 mg/ml could not inhibit all tested pathogens. At the concentration of 50 mg/ml *A B, C* and *D* were found to exhibit little inhibitory effects to *S. aureus,* but none of the effects were found in the others.

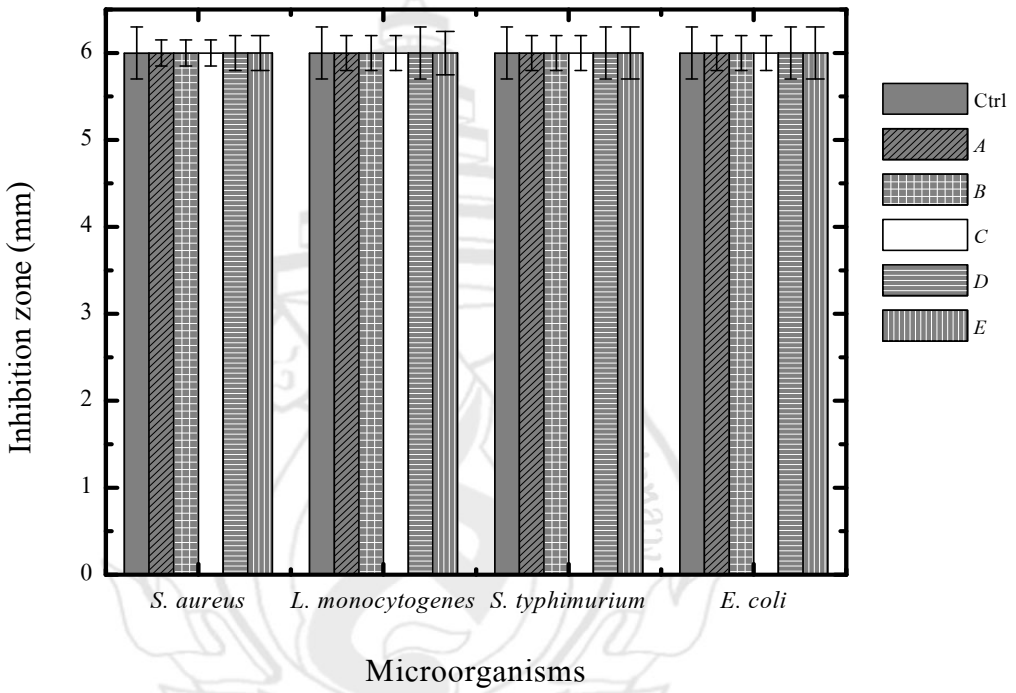


Figure 3.3 Antimicrobial activity observed at 25 mg/ml assam green tea infusion against the tested microorganisms

Figure 3.5, *S. aureus* exhibited high susceptiblity to the assam green tea infusion, compared to *L. monocytogenes, S. typhimurium* and *E. coli. A* at the concentration of 75 mg/ml had highest inhibitory effects, followed by *B, C, D* and *E,* respectively to *S. aureus* and *L. monocytogenes*. At 100 mg/ml, assam green tea infusion inhibited the gram positive bacteria, *S. aureus* and *L. monocytogenes,* as shown in Figure 3.6. However, the less inhibitory effect of 100 mg/ml assam green tea infusion was observed in tea infusion against *S. typhimurium.* Among all of assam green tea infusion, only *A* showed the inhibitory effect against this bacteria. *E. coli* was the most tolerated gram negative bacteria to all assam green tea infusions.

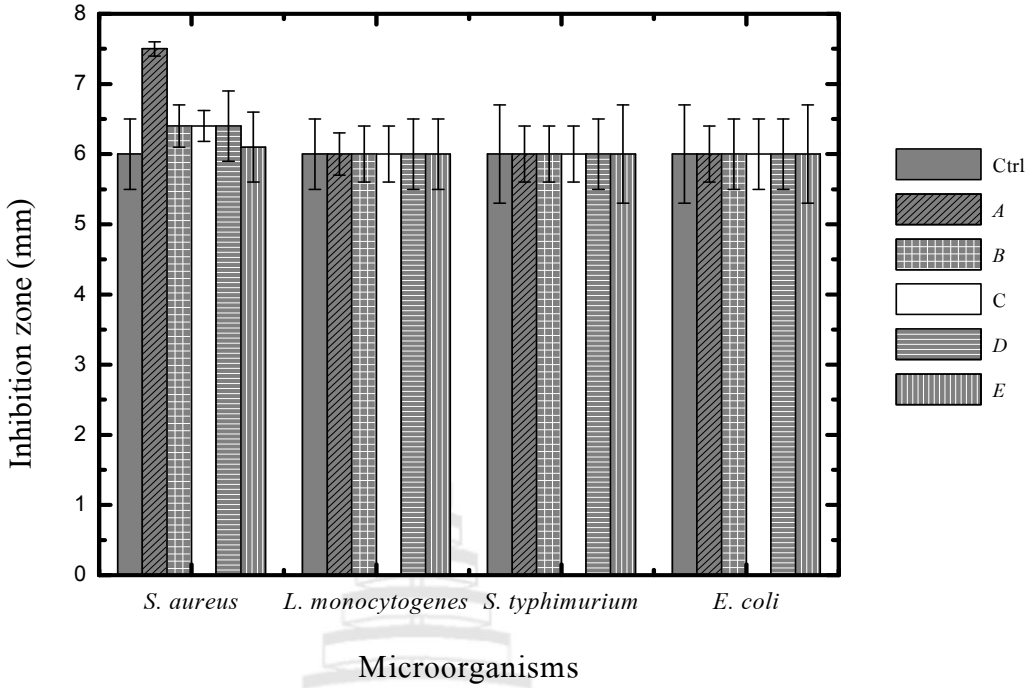


Figure 3.4 Antimicrobial activity observed at 50 mg/ml assam green tea infusion against the tested microorganisms

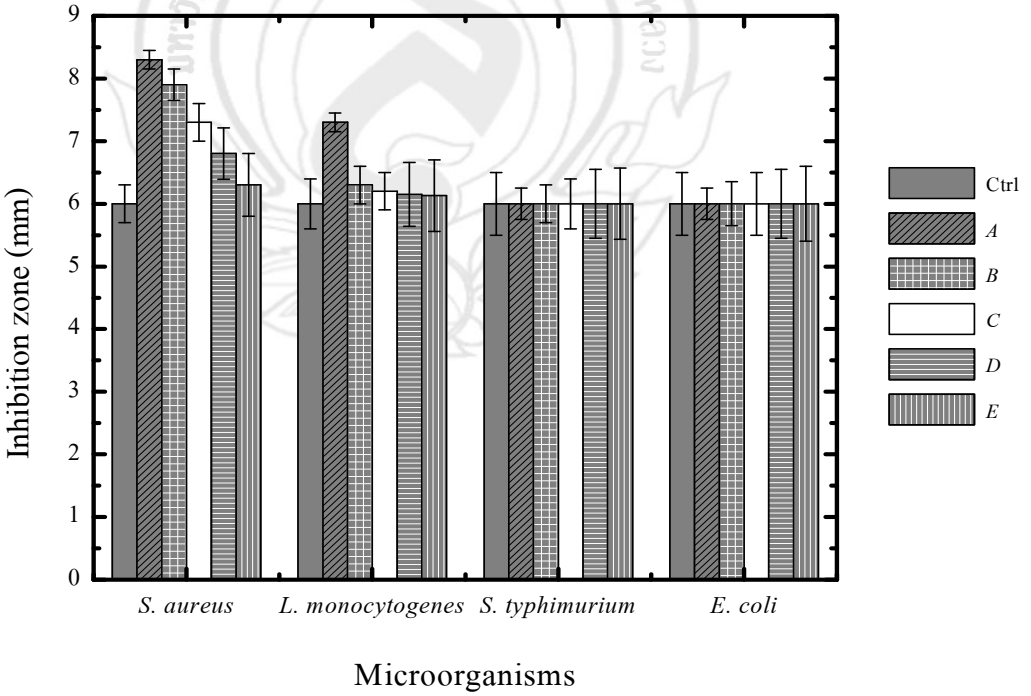


Figure 3.5 Antimicrobial activity observed at 75 mg/ml assam green tea infusion against the tested microorganisms

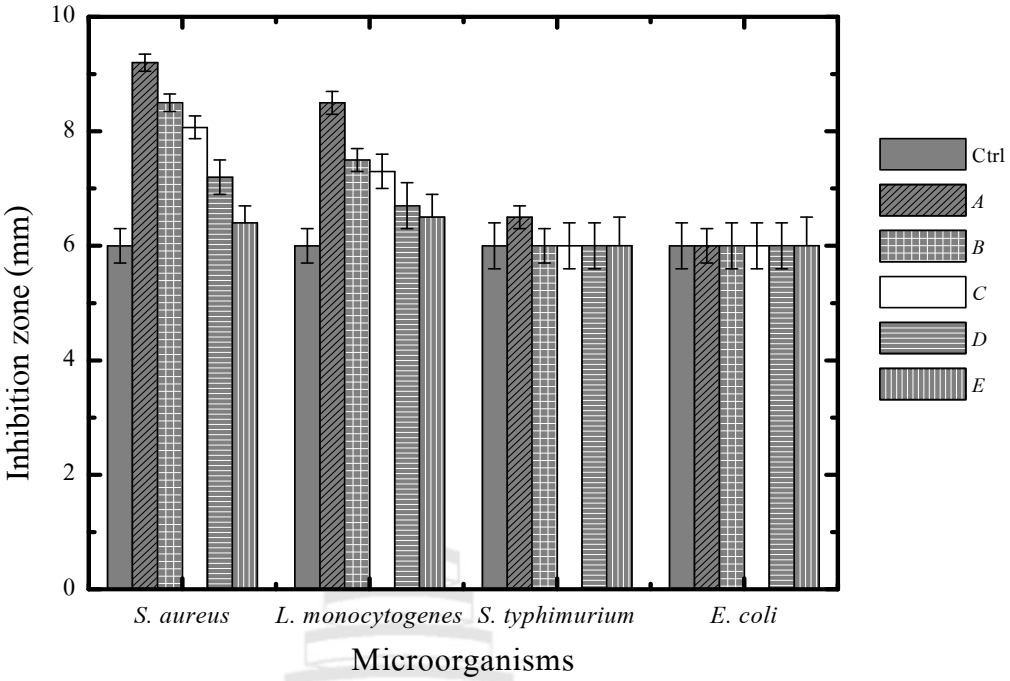


Figure 3.6 Antimicrobial activity observed at 100 mg/ml assam green tea infusion against the tested microorganisms

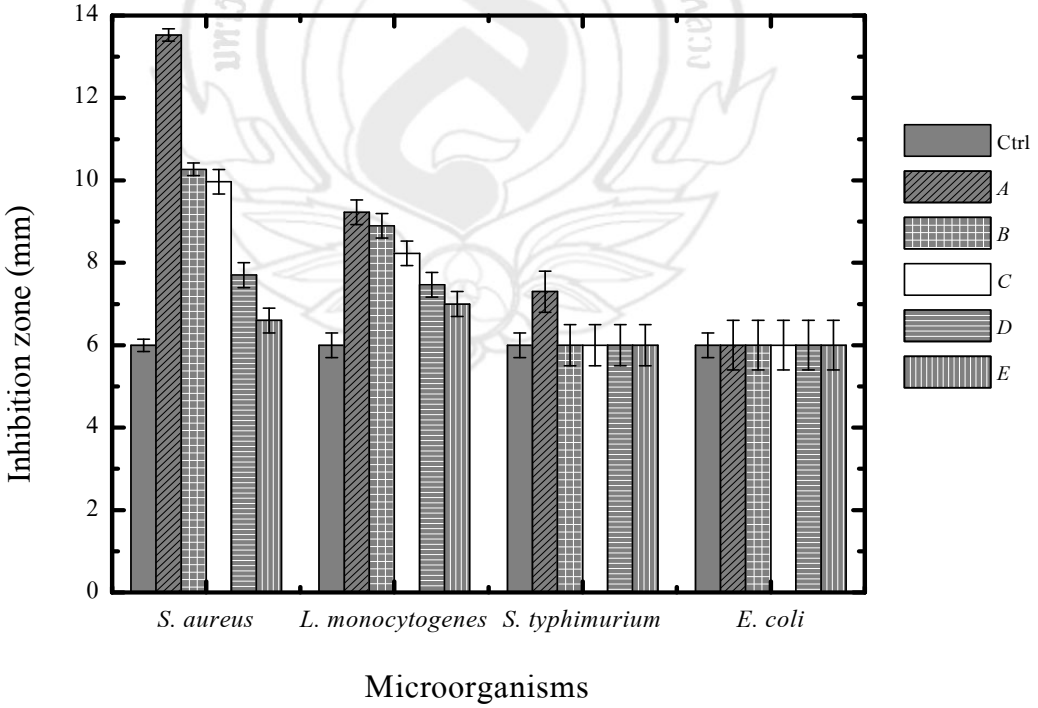


Figure 3.7 Antimicrobial activity observed at 125 mg/ml assam green tea infusion against the tested microorganisms

The same results were observed in assam green tea infusion at the 125 mg/ml. *A* showed the highest ability to inhibit *S. aureus, L. monocytogenes* and *S. typhimurium,* whereas *E. coli* was found to be the most resistant to all commercial assam green tea infusions at the concentration of 125 mg/ml (Figure 3.7). As shown in Figure 3.8, all commercial assam green tea infusions at the concentration of 150 mg/ml was observed to have higher inhibitory effects to gram positive bacteria, indicated by higher inihibition zones observed for *S. aureus* (10.09 ± 2.11 mm) and *L. monocytogenes* (9.34 ± 1.3 mm) compared to gram negative, *S. typhimurium* (6.44 ± 0.98 mm) and *E. coli* (no inhibition).

Figure 3.9 shows the antimicrobial activity obtained at the concentration of 175 mg/ml assam green tea infusion. It was found that *A* had the highest inhibitory effect to *S. aureus* and *L. monocytogenes,* followed by *B, C, D* and *E.* Among gram positive, *S. aureus* was the most susceptible while among gram negative, *E. coli* was the most tolerated to all tea infusion. These results were consistent with all assam green tea infusions at the concentration of 200 mg/ml (Figure 3.10).

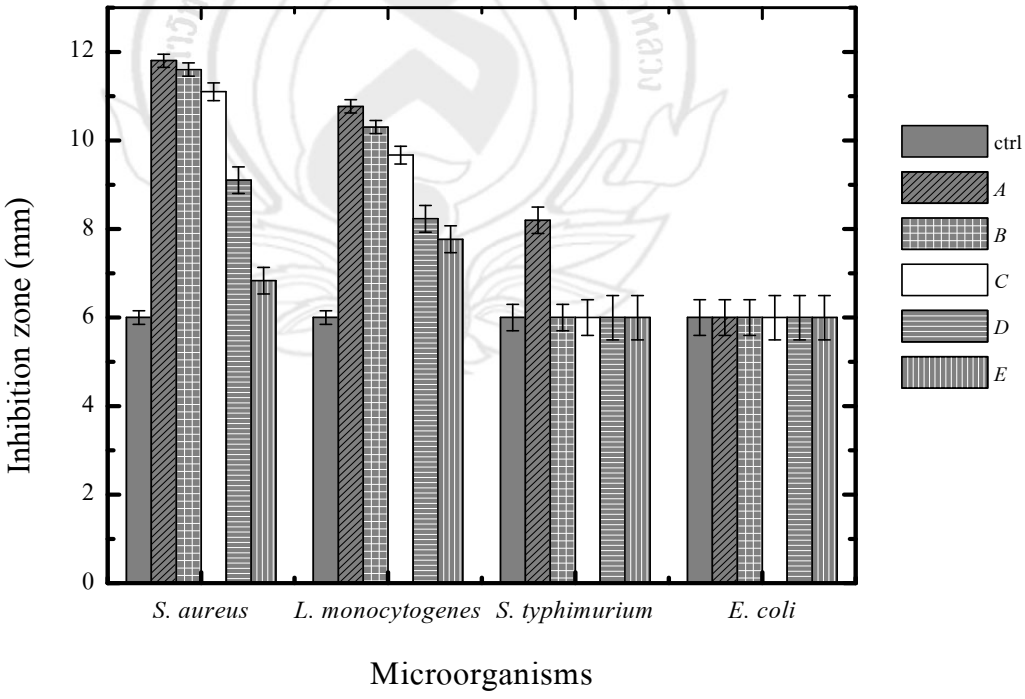


Figure 3.8 Antimicrobial activity observed at 150 mg/ml assam green tea infusion against the tested microorganisms

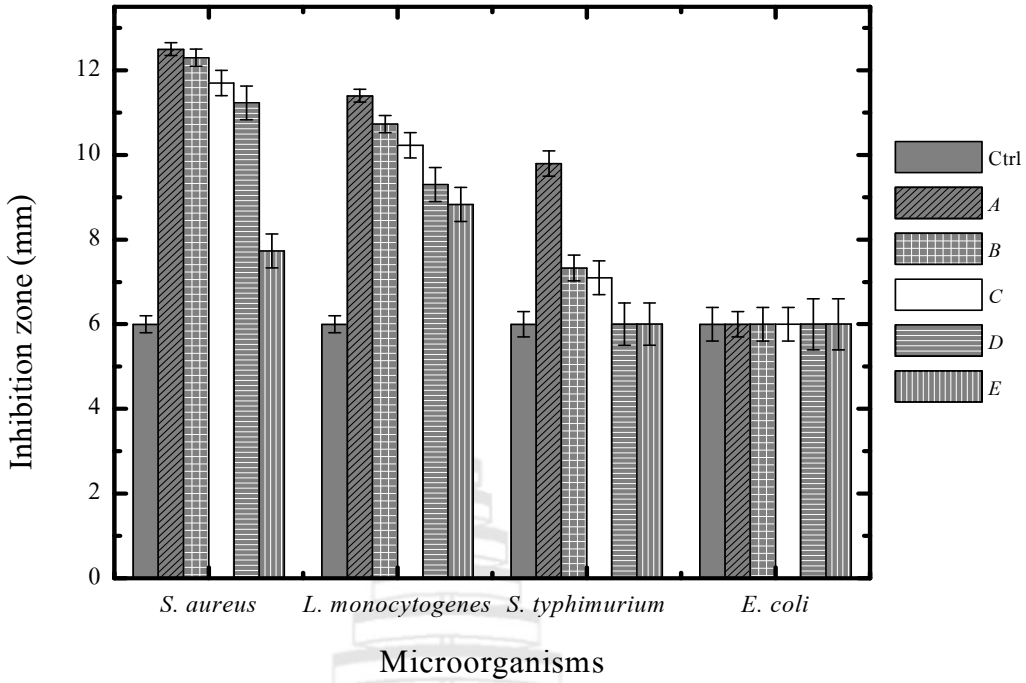


Figure 3.9 Antimicrobial activity observed at 175 mg/ml assam green tea infusion against the tested microorganisms

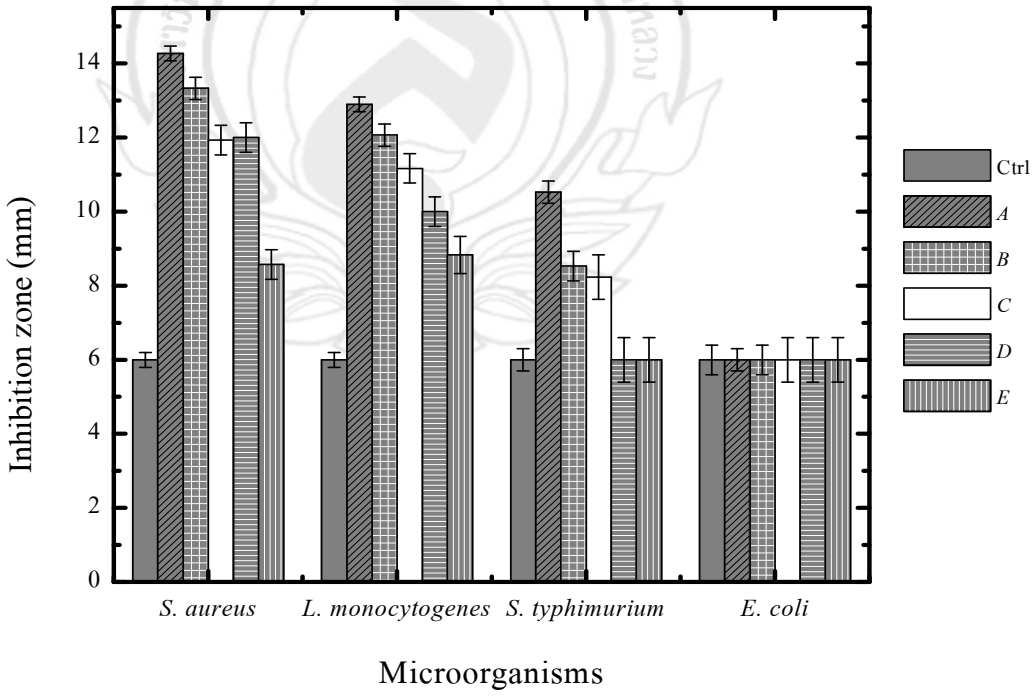


Figure 3.10 Antimicrobial activity observed at 200 mg/ml assam green tea infusion against the tested microorganisms

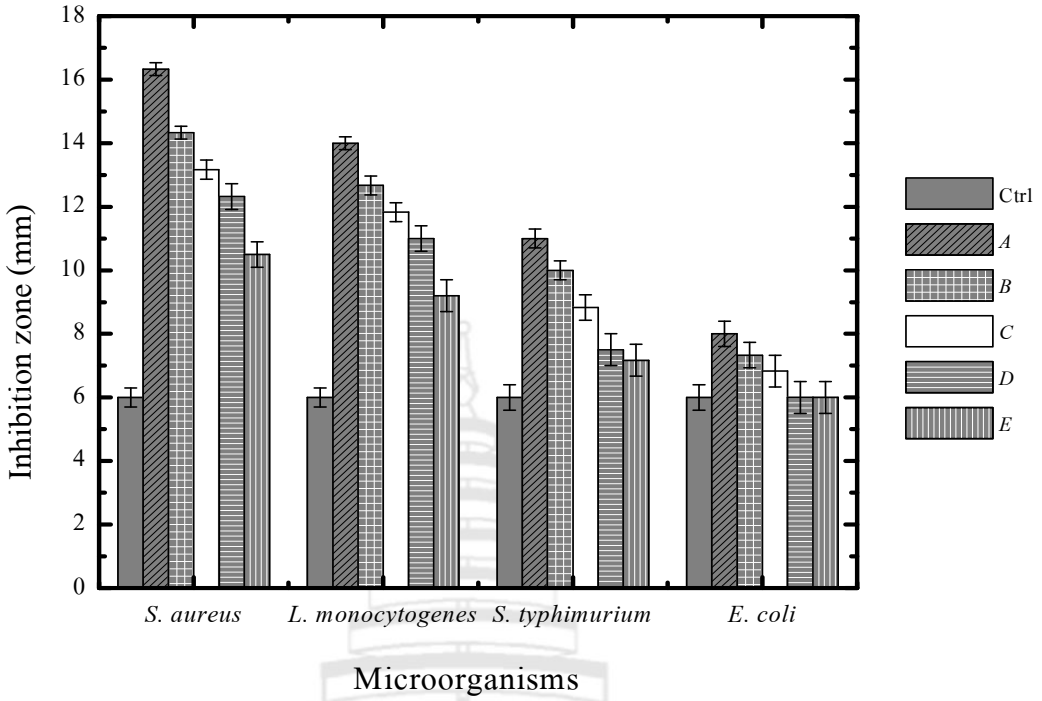


Figure 3.11 Antimicrobial activity observed at 250 mg/ml assam green tea infusion against the tested microorganisms

The antimicrobial activity evaluation at 250 mg/ml assam green tea infusion showed that *A* had the highest inhibitory effect to all tested pathogens, followed by *B, C, D* and *E,* respectively as shown in Figure 3.11 ($p \leq 0.05$). *S. aureus* exhibited to be more susceptible to the assam green tea infusion, compared to *L. monocytogenes, S. typhimurium* and *E. coli*. These results were apparently consistent to the tea TPC and antioxidant activity. In addition, the gram positive bacteria exhibited to be more susceptible to the assam green tea infusions than the gram negative bacteria, which is similar to the results obtained by some previous reports (Cheng-Chun et al., 1999; Fernandez et al., 2005; Tiwari et al., 2005; Almajano et al., 2008; Michalczyk and Zawislak, 2008). These are evidently due to the different cell membrane compositions of both types of bacteria. The absence of lipopolysaccarides in gram positive bacteria results in an alteration of the bacteria membrane structure and cytoplasmic content and consequently causes cell death (Kajiya et al., 2002; Kumazawa et al., 2004). The presence of lipopolysaccharides in outer membranes of gram negative bacteria results in loss of antimicrobial activity of tea

catechins (Kajiya et al., 2004). Among the gram positive bacteria, *S. aureus* exerted less durability to the assam green tea infusions than *L. monocytogenes*. In the case of gram negative bacteria, *E. coli* can survive in the tea infusions better than *S. typhimurium* as indicated by the lower inhibitory effect of *E. coli* (6.83 ± 0.87 mm) compared to that of *S. typhimurium* (8.9 ± 1.63 mm).

The different MICs against the tested bacteria of assam green tea infusions are listed in Table 3.4. The results showed that MICs of all assam green tea infusions exhibited similar tendency for all the tested microorganisms. *S. aureus* exhibited the least resistance in the assam green tea infusion, followed by *L. monocytogenes, S. typhimurium* and *E. coli*, respectively. These results revealed that *S. typhimurium* needs less or the same tea concentration for *E. coli* to illustrate the first observable inhibitory effect. For instance, only 100 mg assam green tea/ml was able to inhibit *S. typhimurium*, whereas double the tea concentration is needed for the inhibitory effect to be observed for *E. coli*.

The inhibitory effect of green tea was also observed by many researchers (Yam et al., 1997; Kim et al., 2004). However, there are some contrary reports by some groups about the antimicrobial activity of tea (Ryu et al., 1982; Toda et al., 1989; Miller, 1995; Tiwari et al., 2005). These must be due to the different varieties of green tea, processing, extraction methods and the available active compounds. It is noteworthy that *A* and *B* had high antimicrobial activity. *A* and *B* also had high EC and EGC content. These indicate that the inhibitory effect of the assam green tea infusions were responsible by combination of EC and EGC content. These results are consistent with Zhang et al. (1997), who reported that among all of the individual catechins, EC and EGC provide the highest antimicrobial activity. According to Table 3.3, it can be observed that *A* and *B* contain mainly EC and EGC, while *E* apparently contains the least of these main catechins, as well as the least TPC, antioxidant and antimicrobial activities.

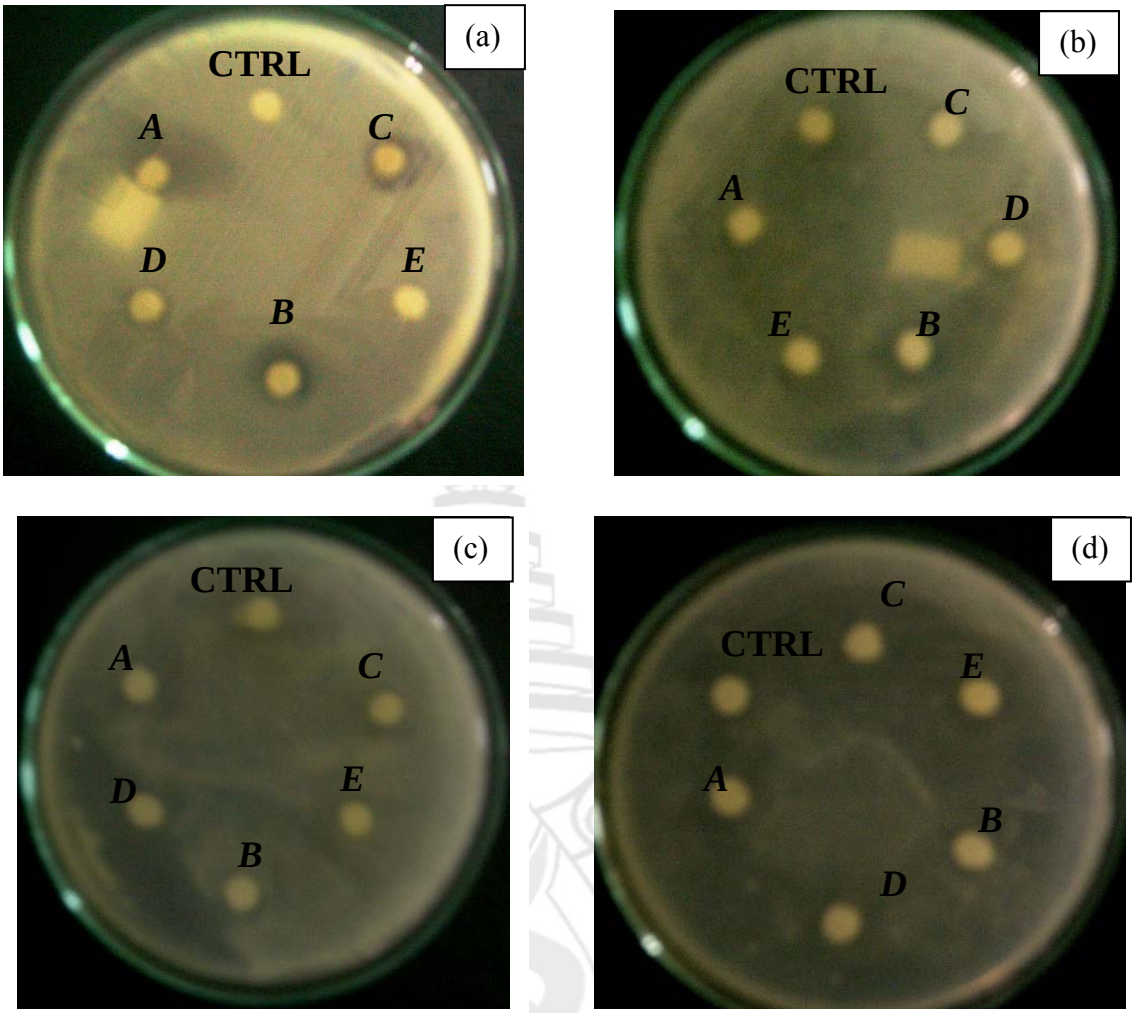


Figure 3.12 Clear zone observed at 250 mg/ml assam green tea infusion for (a) *S. aureus* (b) *L. monocytogenes* (c) *S. typhimurium* and (d) *E. coli*

Table 3.4 MICs of assam green tea infusion against the tested microorganisms

Sample	MICs* (mg/ml)			
	S. aureus	*L. monocytogenes*	*E. coli*	*S. typhimurium*
A	50	75	200	100
B	50	75	200	150
C	50	75	200	150
D	50	75	200	200
E	50	75	200	200

*MIC : the lowest concentration that the first inhibitory effect observed

3.4 Conclusion

The quality of assam green teas available in Chiang Rai, in terms of antioxidant and antimicrobial activities, was found to be different. The significant differences was observed at the highest concentration of the assam green tea infusion (250 mg/ml). The TPC, antioxidant activity and antimicrobial activity all exhibited the same tendency. The most effective antioxidant and antimicrobial assam green tea infusion was significantly observed in *A* and followed by *B, C, D* and *E,* respectively. The assam green tea infusion showing the most effective antioxidant and antimicrobial properties are composed of two main bioactive compounds, EGC and EC. This indicates that those compounds contribute to those properties. Chiang Rai assam green tea infusion exerted the powerful properties, which could be alternately applied in food so as to stabilize the product regarding to their oxidative and microbial susceptibility.

CHAPTER 4

ANTIMICROBIAL ACTIVITY OF COMMERCIAL ASSAM GREEN TEA INFUSION IN LIQUID MEDIUM

4.1 Introduction

There are many investigations *in vitro* of tea antimicrobial properties (Sakanaka et al., 1989; Sakanaka et al., 2000; Su et al., 2008). However, many factors can influence these properties including food compositions and food matrix (Spencer, 2001). These may be due to the combination of food compounds, which may enhance or impair the antimicrobial properties of tea.

There are many types of foods available in liquid form. These include milk, fruit juice and other beverages. These foods are always consumed fresh or partially processed. Therefore, this chapter is aimed to apply the antimicrobial acivity of the assam green tea infusion obtained from Chapter 3 to liquid medium in order to mimic the liquid food matrix, which will further be subjected to the real liquid food matrix in the next chapter.

4.2 Experimental Procedure

4.2.1 Preparation of Assam Green Tea Infusion

The assam green tea infusion was prepared using the same method as mentioned in Chapter 3. Then, various volumes of the 250 mg/ml assam green tea infusion were throughly mixed with different volumes of distilled water and 0.749 g of brain heart infusion (BHI) powder in order to get the required concentration (0, 25, 50, 75, 100, 125, 150, 175, 200, 225 and 250

mg/ml) as shown in Table 4.1. Then, the assam green tea infusion-BHI mixture was sterilized by passing through a membrane filter with the pore size of 0.45 μm.

4.2.2 Microorganisms Preparation

Bacterial culture was prepared according to the method of Kim and Fung (2004). Briefly, the active bacteria was transferred to 100 ml of brain heart infusion (BHI) broth and incubated at 37 °C for 24 hrs. The microbial concentration of the obtained culture was determined and diluted with 0.1% peptone water in order to get a final concentration of approximately 9.0 log CFU/ml.

Table 4.1 Assam green tea infusion preparation

BHI (g)	Volume of stock 250 mg/ml tea infusion (ml)	Distilled water (ml)	Tea final concentration (mg/ml)
1.48	0	20	0
1.48	2	18	25
1.48	4	16	50
1.48	6	14	75
1.48	8	12	100
1.48	10	10	125
1.48	12	8	150
1.48	14	6	175
1.48	16	4	200
1.48	18	2	225
1.48	20	0	250

4.2.3 Minimum Inhibitory Concentration (MIC) Determination

The MIC in this chapter was defined as the minimum concentration of the tea infusion where there is no visible growth (turbidity) of the microorganisms in the broth observed. The MIC of the green tea infusion against the pathogenic bacteria was determined by a macrodillution broth method (Nasrolahi, 2007) using brain heart infusion (BHI). Briefly, 20 μl

bacterial suspensions of 9.0 log CFU/ml was inoculated into the test tube containing 20 ml of a sterile assam green tea infusion-BHI mixture. Subsequently, the sterile assam green tea infusion-BHI mixture was incubated at 35 °C for 24 hrs.

4.3 Results and Discussion

The reduction of the microbial population in different concentrations of assam green tea infusion are shown in Figure 4.1 to 4.4. Figure 4.5 shows images of different concentrations of assam green tea infusions containing different microorganism cultures after 24 hrs of incubation at 35 °C. Table 4.2 shows the minimum concentrations (MICs) of assam green tea infusion against the tested microorganisms. After 24 hrs, *A* and *B* at the lowest concentration assam green tea infusion of 25 mg/ml significantly ($p \leq 0.05$) reduced *S. aureus* to about 7 log cycle (from 8 log CFU/ml to 0.75 and 0.85 log CFU/ml for *A* and *B*, respectively). *S. aureus* was completely inhibited at 50 mg/ml of both tea infusions. Meanwhile, less inhibitory effects were observed in *C, D* and *E,* indicating by only 4-5 log CFU microbial population reduction at 24 hrs of incubation. The same tendency was also observed in *L. monocytogenes,* but higher MICs were observed for all assam green tea infusions. As show in Table 4.2, the MICs of *A* and *B* for *S. typhimurium* and *E. coli* were the same; 150 and 225 mg/ml, respectively. These are six and nine times respectively of the *A* and *B* concentration needed to completely inhibit *S. aureus.* These indicate that gram-positive bacteria is more susceptible to assam green tea infusion than gram-negative bacteria, which is consistent with the results obtained in Chapter 3.

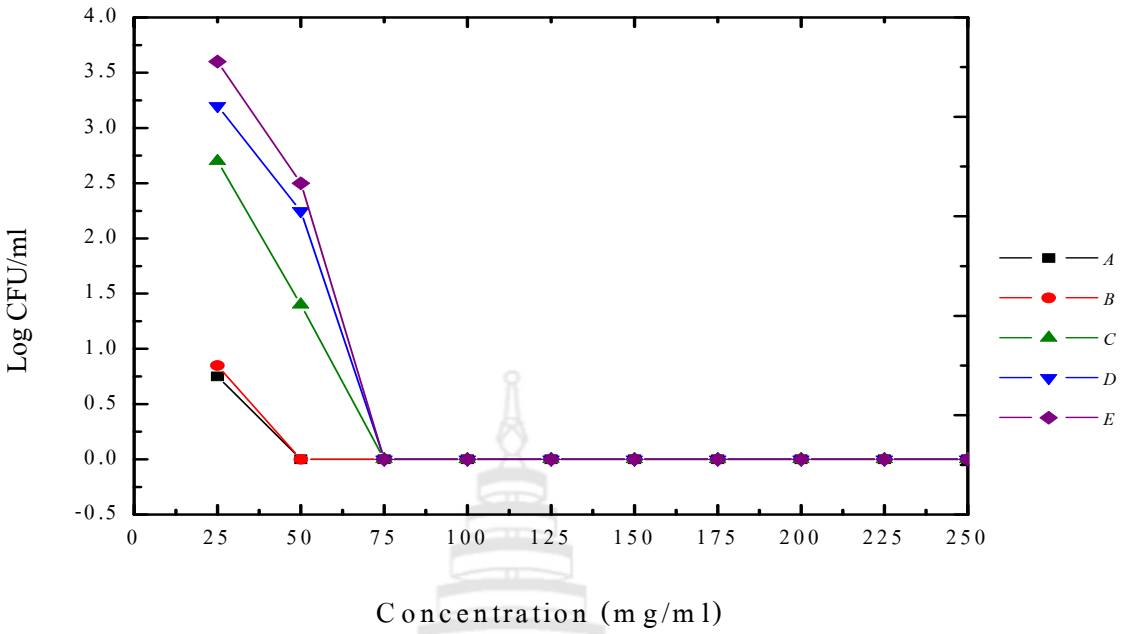


Figure 4.1 *S. aureus* population in different assam green tea infusion concentrations after 24 hrs of incubation at 35 °C with the initial inoculation size of 8 log CFU/ml

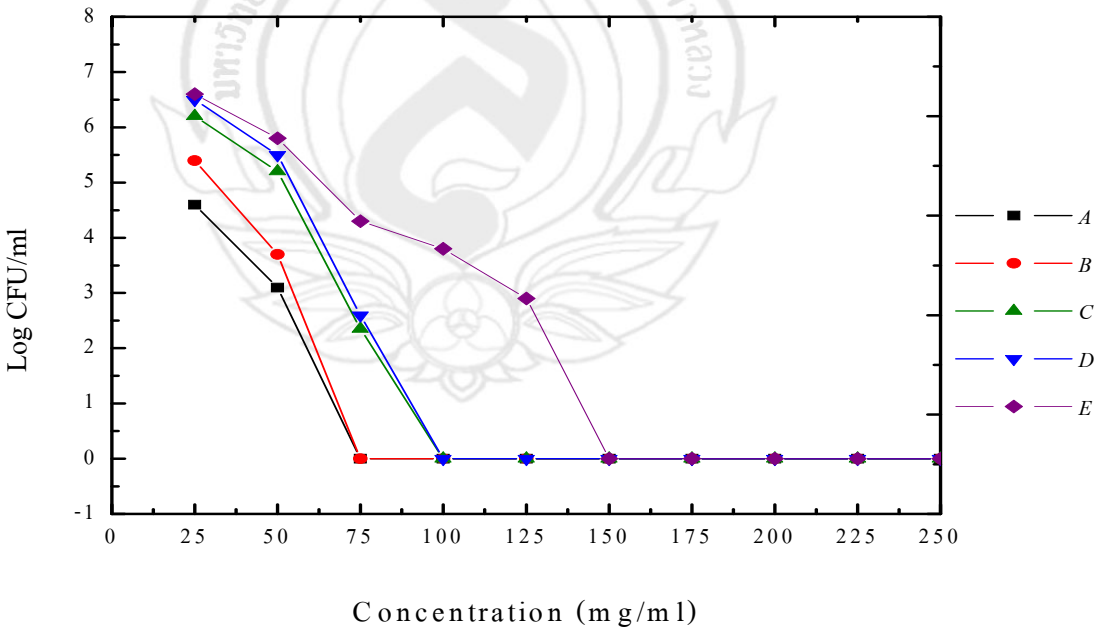


Figure 4.2 *L. monocytogenes* population in different assam green tea infusion concentrations after 24 hrs of incubation at 35 °C with the initial inoculation size of 8 log CFU/ml

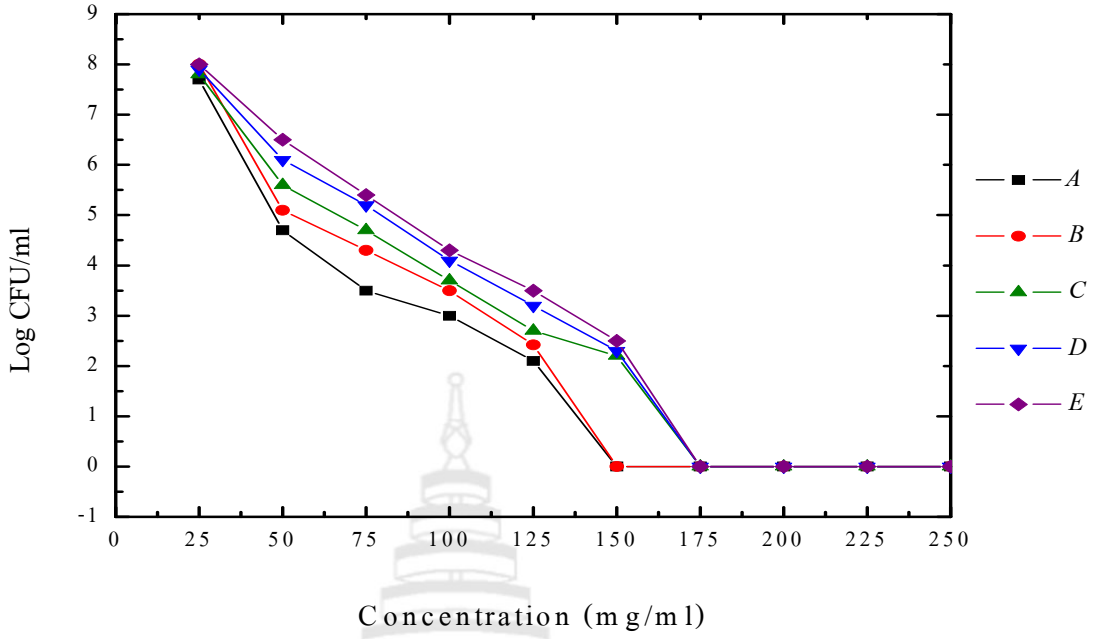


Figure 4.3 *S. typhimurium* population in different assam green tea infusion concentrations after 24 hrs of incubation at 35 °C with the initial inoculation size of 8 log CFU/ml

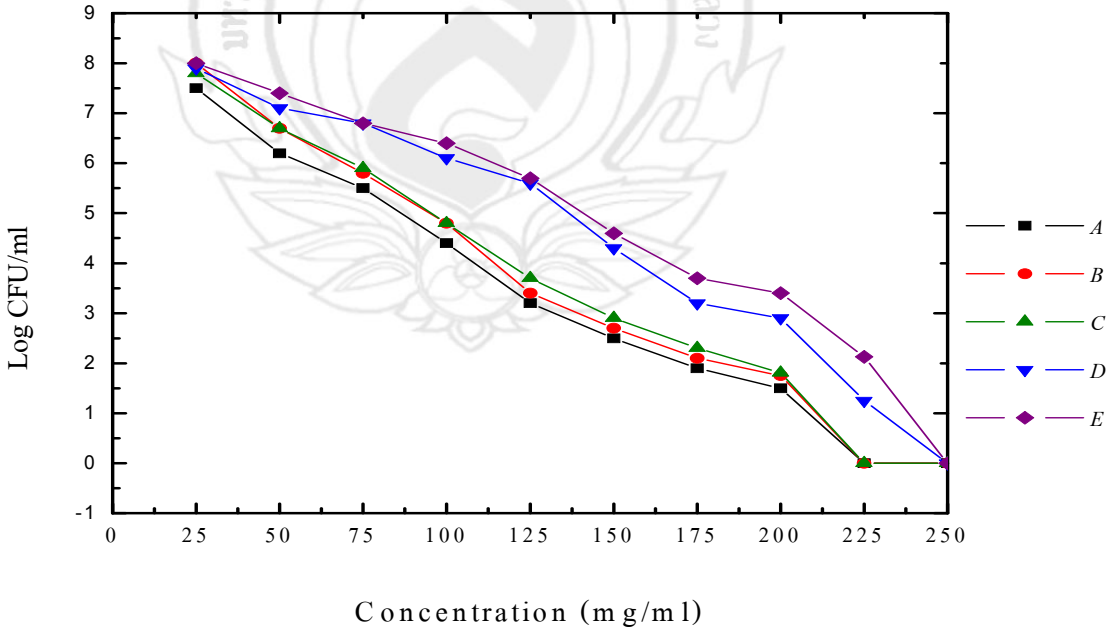


Figure 4.4 *E. coli* population in different assam green tea infusion concentrations after 24 hrs of incubation at 35 °C with the initial inoculation size of 8 log CFU/ml

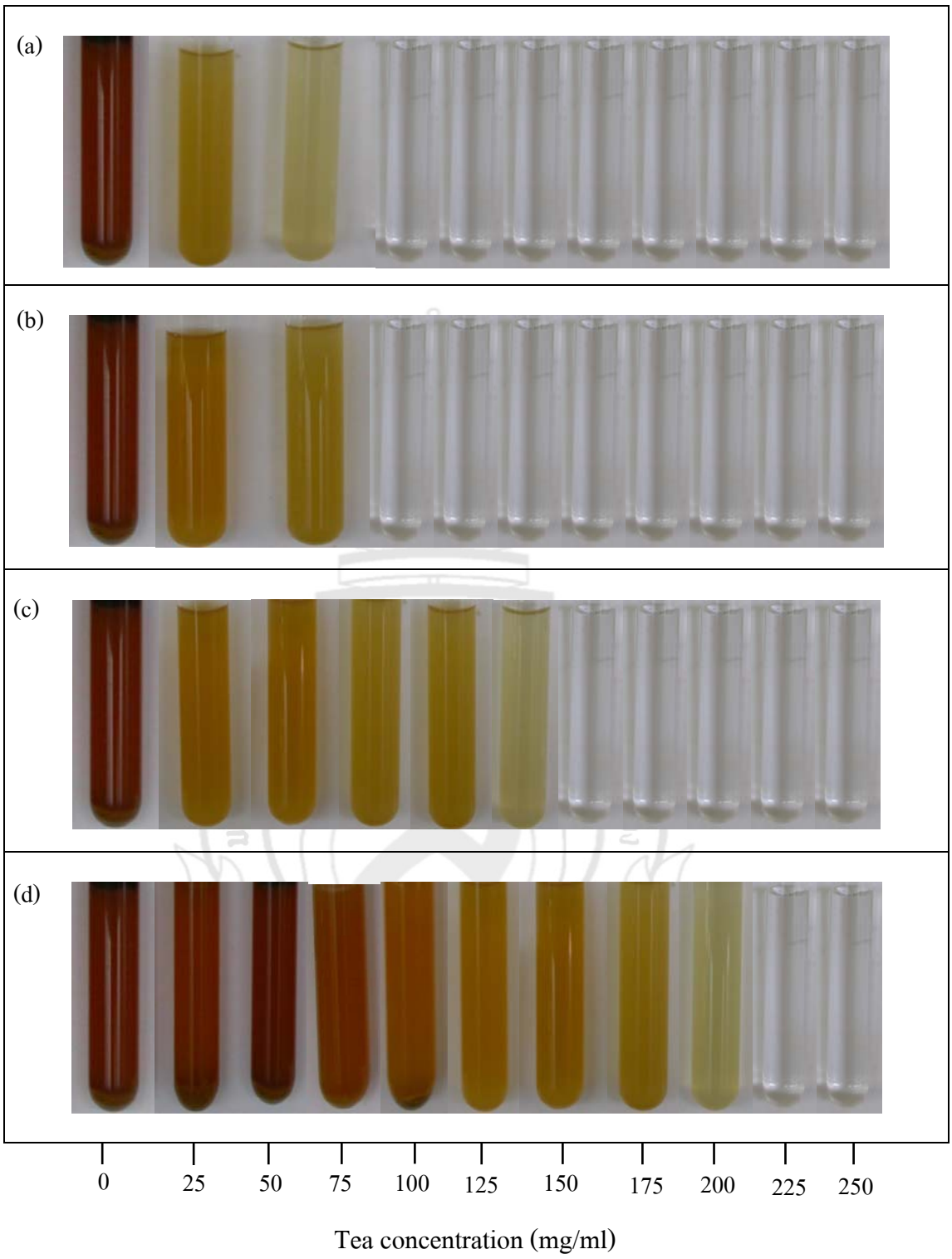


Figure 4.5 Images of different concentrations of assam green tea infusion containing (a) *S. aureus,* (b) *L. monocytogenes,* (c) *S. typhimurium* and (d) *E. coli* after 24 hrs of incubation at 35 °C

Table 4.2 MICs of assam green tea infusions against the tested microorganisms

Samples	MICs* (mg/ml)			
	S. aureus	*L. monocytogenes*	*S. typhimurium*	*E. coli*
A	50	75	150	225
B	50	75	150	225
C	75	100	175	225
D	75	100	175	250
E	75	150	175	250

*MIC : the minimum concentration of the assam green tea infusion where there is no visible growth of the microorganisms

Even though, all of the assam green teas are produced in Chiang Rai, the detailed processes and storage conditions are different. Therefore the qualities, particularly their active components, were significantly different ($p \leq 0.05$) as obtained in this study. These antimicrobial activities of the assam green tea infusion were evidently responsible by its active components as previously reported (Hu et al., 2001; Kajiya et al., 2004; Tiwari et al., 2005; Almajano et al., 2008; Michalczyk and Zawislak, 2008). In addition, these properties were varied depending on the tea variety, extraction method and processing (Wang et al., 2000; Fernandez et al., 2002; Cheng et al., 2005).

4.4 Conclusion

There was significant difference among the commercial assam green tea antimicrobial activity in the liquid medium. The antimicrobial activity of assam green tea infusion in the liquid medium showed the same tendency as observed in the agar diffusion method (Chapter 3). *A* and *B* exhibited significantly higher antimicrobial activity than *C, D* and *E* ($p \leq 0.05$). Therefore, assam green tea *A* and *B* at the concentration of 75, 100, 175 and 250 mg/ml in the mimic liquid medium were applied to investigate again these properties in watermelon juice for the next experiment.

CHAPTER 5

THE EFFECT OF COMMERCIAL ASSAM GREEN TEA INFUSION ON MICROBIAL GROWTH IN WATERMELON JUICE

5.1 Introduction

The consumption of fresh-cut and fruit juices is increasing due to their freshness, high vitamin content and health components (Ashurst, 2005; Berryman, 2007; Rico et al., 2007). However, various outbreaks have been reported to be associated with fresh-cuts and fruit juices (Ray, 2001; Yuste and Fung, 2002; CDC, 2007; Oussalah, 2007; Raybaudi-Massilia et al., 2008). Those outbreaks are mainly caused by *E. coli,* different serovars of *Salmonella, S. aureus, L. monocytogenes,* and *Yersinia enterocolitica*.

Watermelon acidity is very close to neutral (pH 5.2 to 6.7) (FDA, 2001; Mosqueda-Melgar et al., 2007). Therefore, it is a good vehicle for foodborne microorganisms. The FDA (2001) and CDC (2007) reported outbreaks of watermelon juice by *E. coli, S. typhimurium, L. monocytogenes* and *S. aureus.*

As a result of its high nutrition and low acidity possing its high microbial susceptibility than other general high pH fruit juice, therefore watermelon juice was chosen in this experiment as a representative for other fruit juices. *A* and *B* tea infusion that exhibited the most powerful antimicrobial as observed and described in Chapter 3 and 4, were selected to investigate the properties in the liquid model food matrix (watermelon juice), in order to observe the possibility of using this assam green tea infusion in other types of fruit juice.

5.2 Experimental Procedure

Sterile 250 mg/ml assam green tea infusion and 9 log CFU/ml cultures were prepared by the same method used in Chapter 3.

5.2.1 Watermelon Juice Preparation

Watermelon red cultivar was purchased from a supermarket in Chiang Rai, Thailand in the year 2008. The watermelon was washed, peeled and cut into pieces. Then, juice was prepared by blending the watermelon flesh using a blender. The blended flesh was filtered by four layers of sheet clothes to obtain the clear juice. The filtered juice was centrifuged at 10,000 rpm for 15 min at 4 °C. The supernatant of the juice was collected and autoclaved at 121 °C for 15 min. Then, the total soluble solid (TSS) and pH were determined.

5.2.2 Preparation of Tea-Watermelon Juice Mixture and Inoculation

Different concentrations of assam green tea infusion (0, 75, 100, 175 and 250 mg/ml) were prepared by aseptically mixing different volumes of the sterile stock assam green tea infusion and the autoclaved watermelon juice as shown in Table 5.1. The watermelon juice (20 ml) containing different concentrations of assam green tea infusions were inoculated with 0.2 ml of 9 log CFU/ml bacteria suspension to obtain approximately 8 log CFU/ml bacterial concentration. A control watermelon juice without assam green tea infusion, was also inoculated. All of the samples were incubated at 35 °C for 7 days.

5.2.3 Microbiological Analysis

The microbiological analysis was determined everyday up to 7 days. On each day, a serial dillution was prepared in sterile 0.1% peptone water and 0.1 ml of appropriate dilluent was spread into duplicates on culture media to determine the population of *S. aureus, L. monocytogenes, S. typhimurium and E. coli,* respectively. All plates were incubated at 35 °C for 24 hrs.

Table 5.1 Tea-watermelon juice mixture preparation

Volume of stock 250 mg/ml tea infusion (ml)	Autoclaved watermelon juice (ml)	Tea final concentration (mg/ml)	Juice (% v/v)
0	20	0	100
6	14	75	70
8	12	100	60
14	6	175	30
20	0	250	0

5.3 Results and Discussion

Table 5.2 shows the TSS and pH of the obtained tea-watermelon juice mixture. It was found that the TSS of watermelon juice containing assam green tea infusion varied from 9 to 12 %. The TSS obtained in the present study is complied with the TSS that allowed for commercial watermelon juice (FAO 2001). The pH of the watermelon juice containing assam green tea infusion was in a range of 5.69–6.44, which was significantly higher than the control ($p \leq 0.05$). There was no significant difference in pH between *A* and *B* infusion at the same concentration. The pH of the watermelon juice decreased when the assam green tea infusion was added. This might be because of the phenolic acid (chlorogenic acid, isoferulic acid and gallic acid) in the tea infusion as also reported by some other groups (Hodgson et al., 2004; Somboonvechakarn, 2007).

Figure 5.1 to 5.4 shows the images of the watermelon juice-tea mixture observed at different days of incubation with pathogens. The reduction of the microbial population in the watermelon juice-tea mixture is shown in Figure 5.5 to 5.8. The results showed that the number of microorganisms in watermelon juice without tea infusion were not significantly different throughout the storage days. Whereas, all microorganisms in watermelon juice containing tea infusion decreased rapidly to undetected levels by 2 to 4 days of incubation. It was found that

addition of *A* infusion in watermelon juice inactivated *S. aureus* and *L. monocytogenes* within 2 and 3 days, respectively. While, *B* infusion, showed less inhibitory effects to *S. aureus* and *L. monocytogenes* by taking 3 and 4 days to exhibit entirely inhibitory effects, respectively. Both assam green tea infusions had the same inhibitory effect to *S. typhimurium* and *E. coli*, requiring only 5 and 6 days to completely inhibit *S. typhimurium* and *E. coli*, respectively. The obtained less inhibition time needed of *A* and *B* tea infusions suggested that gram positive bacteria (*S. aureus* and *L. monocytogenes*) exhibited more sensitive to the assam green tea infusion than gram negative bacteria (*S. typhimurium and E. coli*). These are consistent to the results obtained in the liquid medium.

Table. 5.2 TSS and pH of watermelon juice

Sample	Conc. (mg/ml)	Parameters	
		TSS (%)	pH
Ctrl	-	9^{a}	6.44^{a}
A	75	9^{a}	6.21^{b}
	100	10^{a}	5.99^{c}
	175	11^{a}	5.79^{c}
	250	12^{a}	5.71^{c}
B	75	9^{a}	6.18^{b}
	100	10^{a}	5.95^{c}
	175	11^{a}	5.75^{c}
	250	12^{a}	5.69^{c}

*a-d Means with different superscript letters within a column are significantly different at $p \leq 0.05$

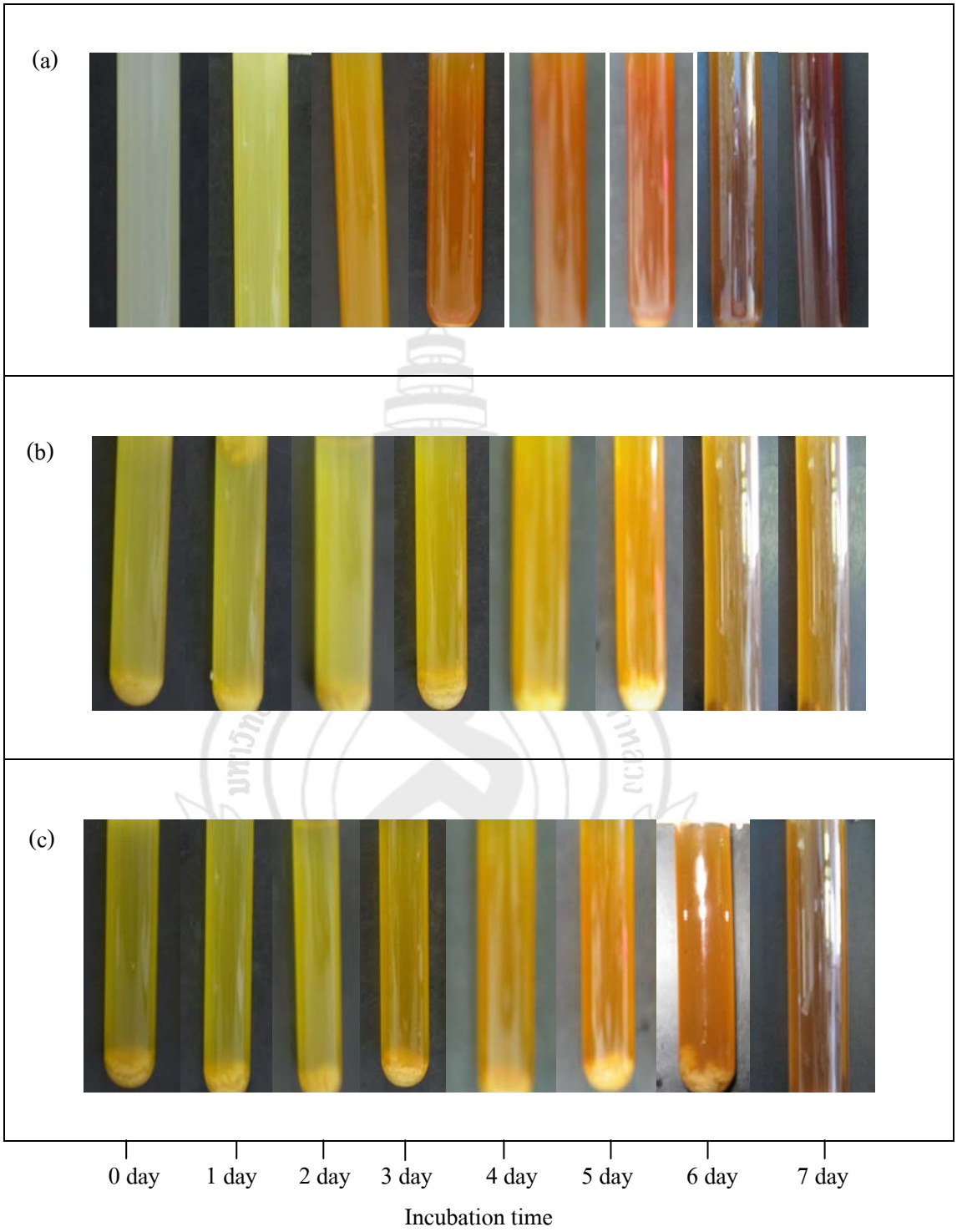


Figure 5.1 Watermelon juice (a) watermelon juice-tea mixture containing 75 mg/ml *A* (b) and 75 mg/ml *B* (c) inoculated with *S. aureus* after 7 days of incubation at 35 °C

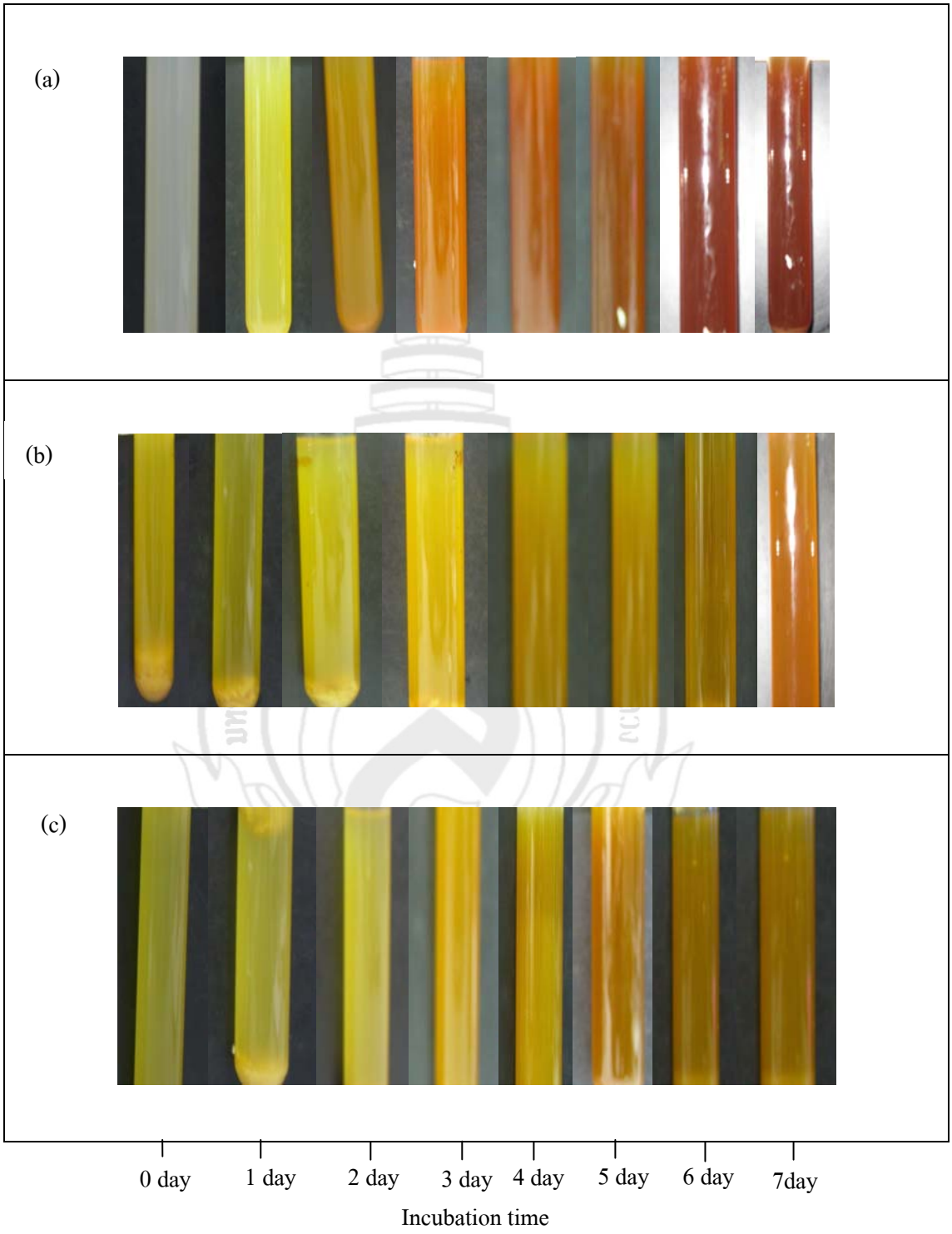


Figure 5.2 Watermelon juice (a) watermelon juice-tea mixture containing 100 mg/ml *A* (b) and 100 mg/ml *B* (c) inoculated with *L. monocytogenes* after 7 days of incubation at 35 °C

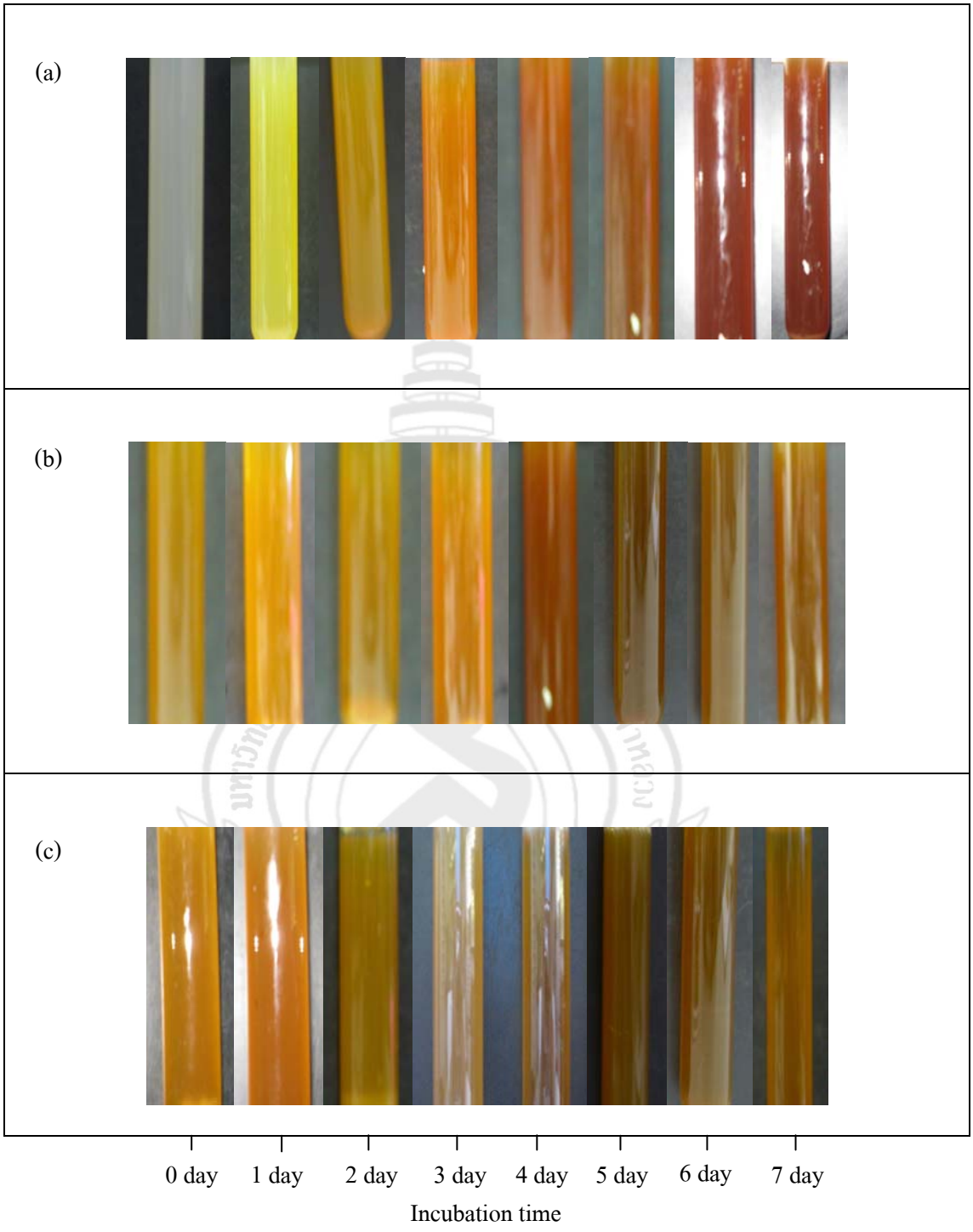


Figure 5.3 Watermelon juice (a) watermelon juice-tea mixture containing 175 mg/ml *A* (b) and 175 mg/ml *B* (c) inoculated with *S. typhimurium* after 7 days of incubation at 35 °C

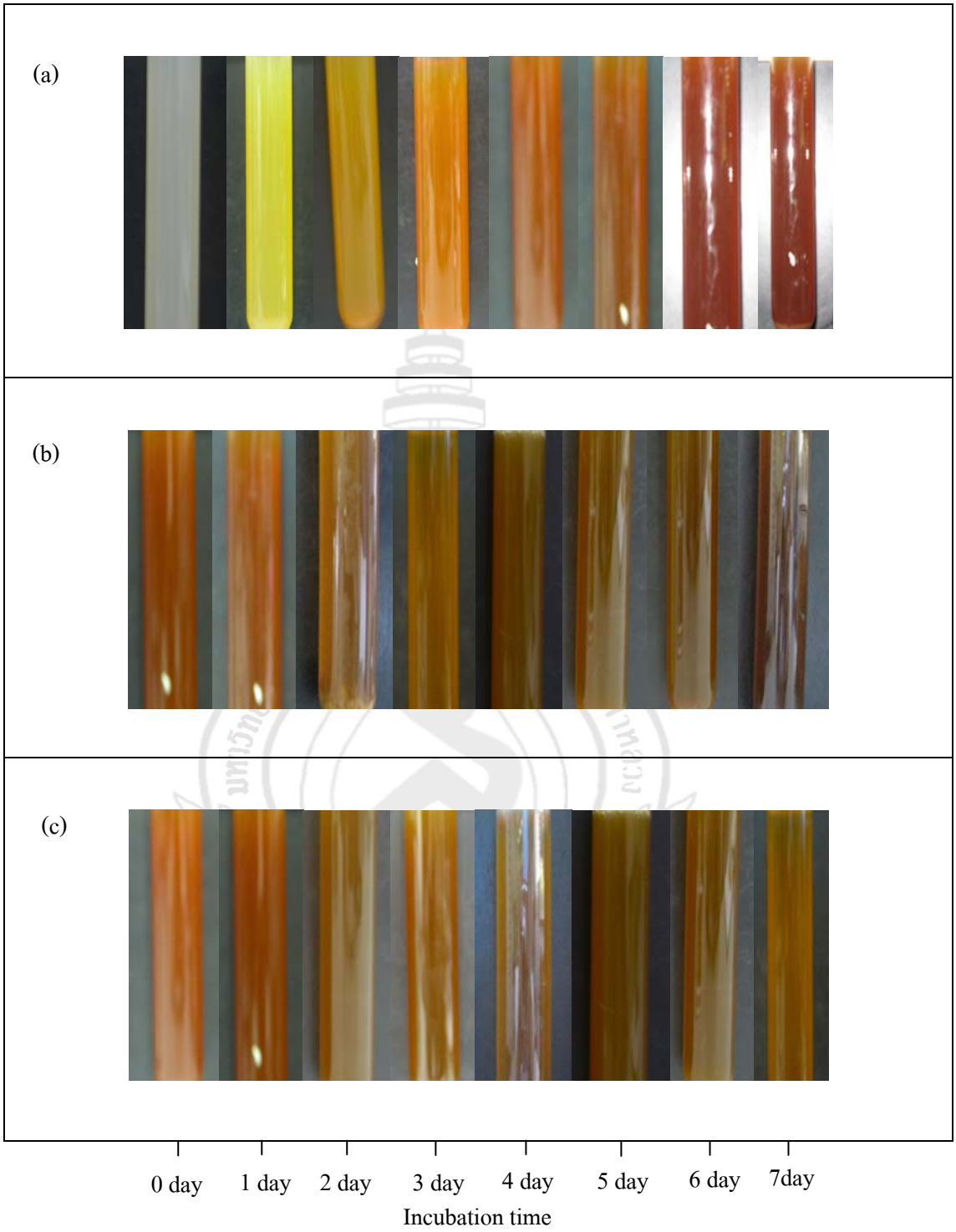


Figure 5.4 Watermelon juice (a) watermelon juice-tea mixture containing 250 mg/ml *A* (b) and 250 mg/ml *B* (c) inoculated with *E. coli* after 7 days of incubation at 35 °C

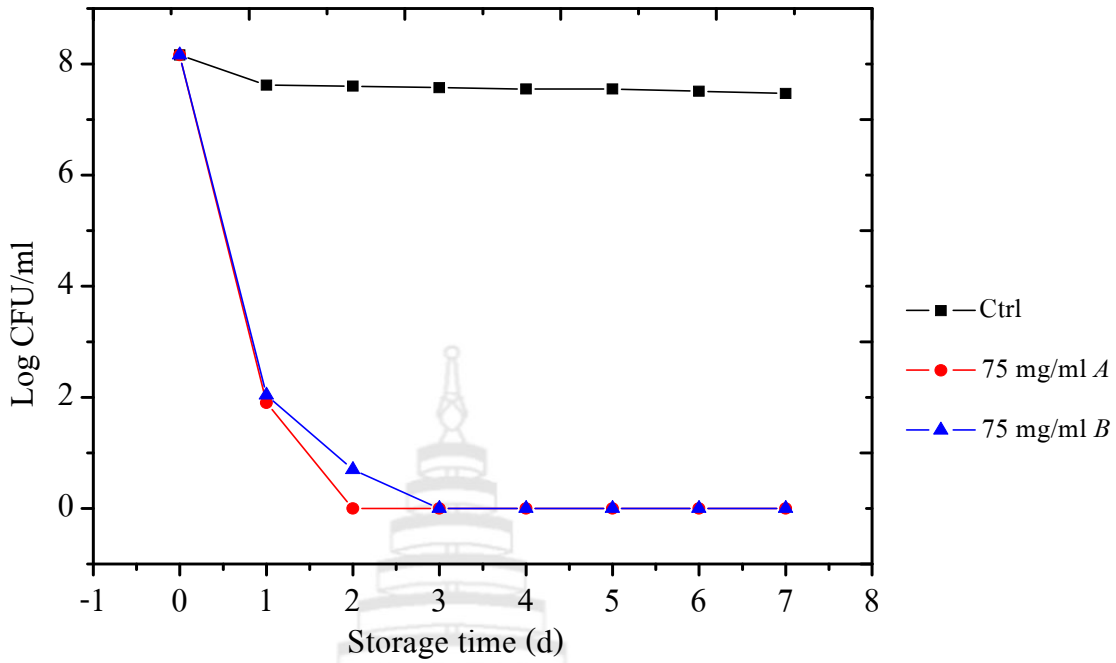


Figure 5.5 Reduction of *S. aureus* in the watermelon juice-tea mixture after 7 days of incubation at 35 °C

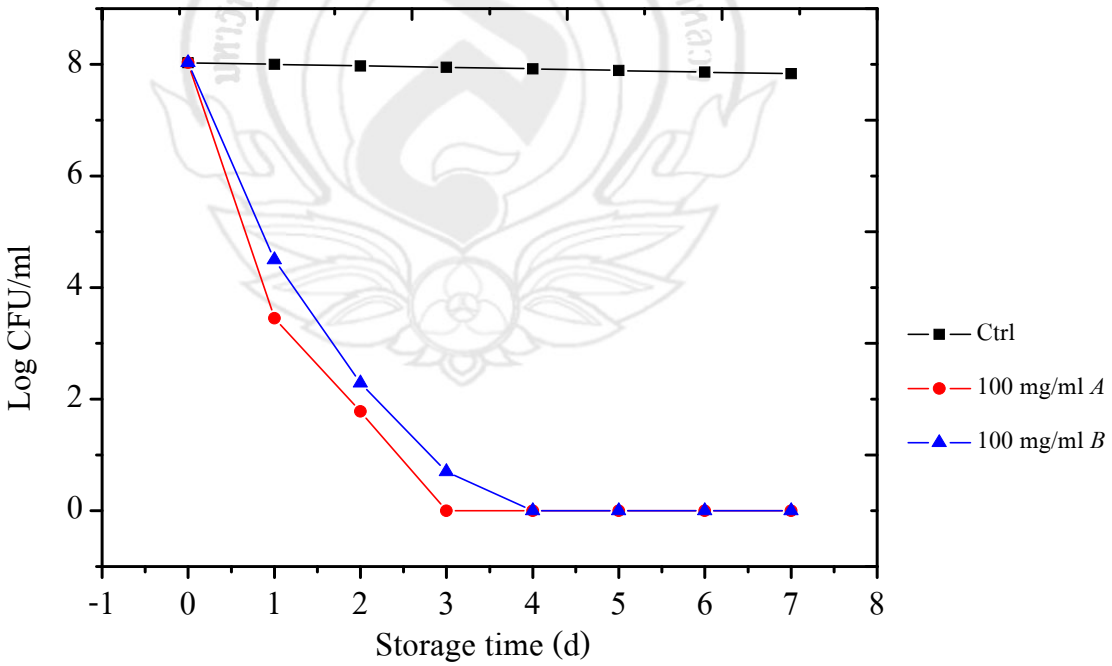


Figure 5.6 Reduction of *L. monocytogenes* in the watermelon juice-tea mixture after 7 days of incubation at 35 °C

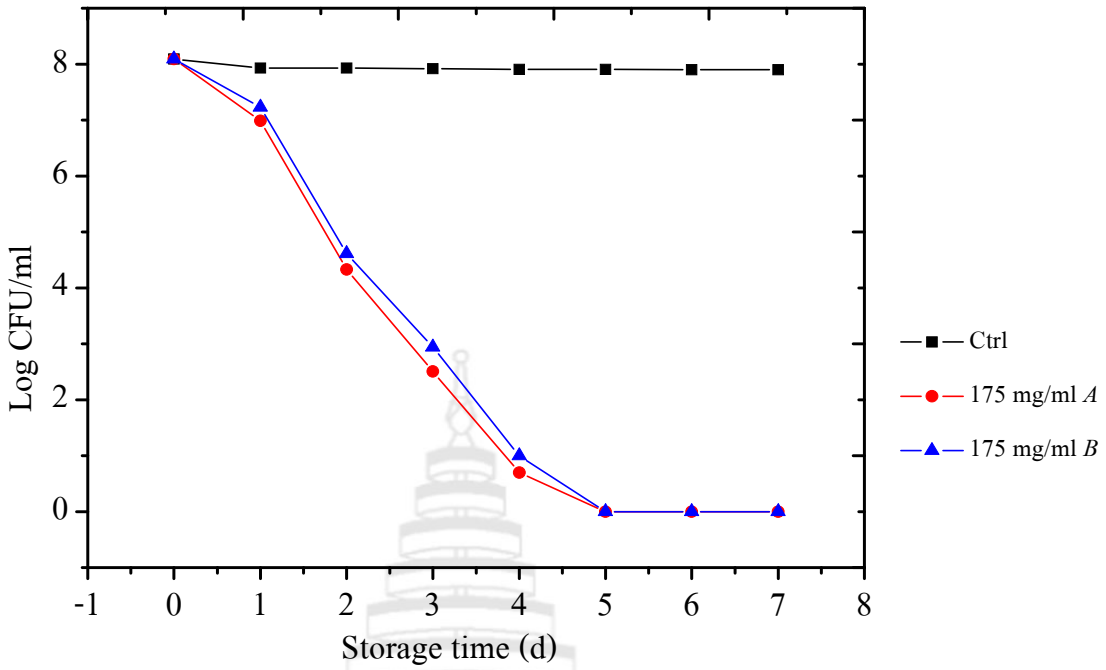


Figure 5.7 Reduction of *S. typhimurium* in the watermelon juice-tea mixture after 7 days of incubation at 35 °C

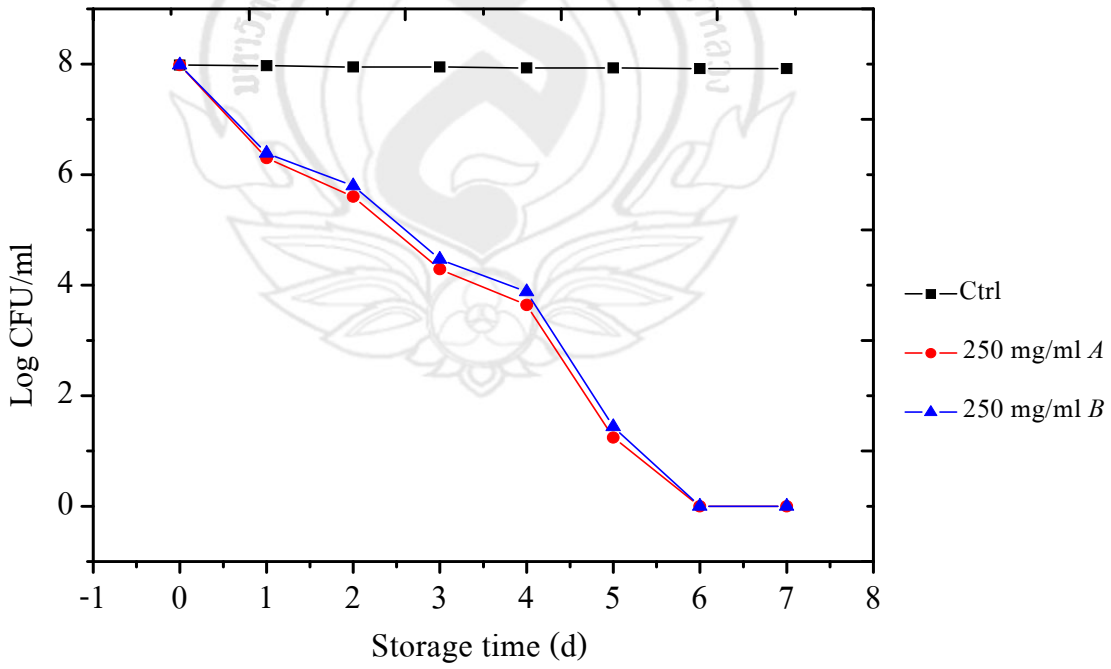


Figure 5.8 Reduction of *E. coli* in the watermelon juice-tea mixture after 7 days of incubation at 35 °C

Bactericidal of green tea in watermelon juice is a consequence of the lower pH in the juice-tea mixture (Burt, 2004). In addition, the hydrophobicity of green tea increased at low pH, enabling it to more easily dissolve in the cytoplasmic content of the target bacteria, hence a greater bactericidal effect was obtained (Raybaudi-Massilia et al., 2008). These suggest that the antimicrobial activity of the green tea was a combination effect of the tea phenolic compounds and the low pH with the tea infusion addition. However, the action of tea infusion against bacteria in watermelon juice was lower than that of the liquid medium. This might be due to a greater availability of nutrients in the watermelon juice compared to the liquid medium, which may enable bacteria to repair damaged cells faster as also reported by other researchers (Gill et al., 2002; Munoz et al., 2009).

5.4 Conclusion

The microbial inhibitory of *A* and *B* at 75 and 100 mg/ml, respectively exhibited the same tendency as observed in the liquid medium against *S. aureus, L. monocytogenes, S. typhimurium and E. coli*. *A* was observed to have the highest inhibitory effect of all pathogens. *E. coli* was the most resistant strain compared to *S. aureus, L. monocytogenes* and *S. typhimurium.* This study showed that green tea infusion can extend the shelf life of watermelon juice up to 7 days. As a result of its almost neutral pH, watermelon juice is more susceptible to microbial spoilage than other fruit juices which are normally acidic. This suggests that tea infusion might be a potential natural preservative used to extend the shelf life of fruit juices and could improve their safety. However, combinations of preservatives can be applied to enhance the antimicrobial activity against the resistant strain *S. typhimurium* and *E. coli.* These combinations of different preservation techniques is the basic hurdle concept to provide fresh-like quality food products.

CHAPTER 6

THE EFFECT OF COMMERCIAL ASSAM GREEN TEA INFUSION ON MICROBIAL GROWTH AND OXIDATIVE STABILITY IN COOKED BEEF

6.1 Introduction

As a result of its high nutrition, beef products are classified as a perishable food. The demand of ready-to-eat food has been increasing over the last decade (Williams, 2007), with requirements for good quality and safety. However, beef contains high iron content, which acts as catalyst in the oxidation reaction (Ho et al., 2009). This has led to high oxidative susceptibility, leading to the deterioration of beef. The deterioration of beef is not only caused by lipid oxidation but also caused by microbial contamination (Ahn et al., 2002). These make beef products unacceptable and unsafe for the consumer, particularly in the new era of health concerned society.

Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) mixtures were reported as effective synthethic food additives to prevent both lipid oxidation and microbial contamination (Tang et al., 2001; Ahn, 2003; Mitsumoto et al., 2005). However, BHA and BHT, like the other synthetic food additives, have a limitation level allowed for food application to not more than 0.01% by weight (FAO, 2006). Therefore, many investigations have been developed in order to find out the natural food additives having the same properties as BHA and BHT to be antioxidant and antimicrobial agents (Ahn, 2003, Aksu and Kaya, 2004).

Tea is one of powerful natural preservative with those two properties. Even though, researchers have been attempting to investigate those properties of tea in various forms including tea extract and tea catechins *in vitro* and in food model systems (Tang et al., 2001; Bong-Jeun et al., 2004, Mitsumoto et al., 2005; Su et al., 2008), there is no report of using tea-water infusion

directly for those purposes in the real food matrix. Therefore, this experiment has applied the results obtained in Chapter 3 to investigate the antimicrobial and anti-lipid oxidation in the food model, cooked beef. These will provide the information of the assam green tea infusion feasibility as antimicrobial and anti-lipid oxidation in other foods.

6.2 Experimental Procedure

Assam green tea and microorganisms were prepared the same as mentioned in Chapter 3. Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were purchased from Sigma-Aldrich Co, USA. Microbiological media, including Modified Oxford (MOX) and Xylose Lysine Deoxycholate (XLD) agar were purchased from Merck, Germany, while petrifilm for *E. coli*/coliform count and *S. aureus* express count plate were purchased from 3M Microbiology Thailand, Ltd.

6.2.1 Assam Green Tea Preparation

Different concentrations of assam green tea infusion were proposed by mixing different volumes of the stock assam green tea infusion (250 mg/ml) and distilled water as shown in Table 6.1 in order to get 0, 31.3, 62.5, 125 and 250 mg/ml assam green tea infusion. Then, the extract was sterilized by passing it through a membrane (pore size of 0.45 μm). In antimicrobial activity determination, three different assam green tea infusion concentrations were used : 0, 125 and 250 mg/ml, while five different concentrations of assam green tea infusion were used to determine anti-lipid oxidation activity in cooked beef. Whereas, synthetic antioxidant, 0.02% of BHA and BHT solution were prepared by mixing 0.01% BHA and 0.01% BHT solution in ethanol.

6.2.2 Microorganism Preparation

Bacterial cultures were transferred to 10 ml brain heart infusion (BHI) broth and incubated at 37 °C for 24 hrs. The cultures were then transferred to 100 ml BHI broth and allowed to grow for 24 hrs. The broth was centrifuged at 5,000 *g* for 15 min at 4 °C. The culture

was then washed and resuspended with 0.1 % peptone water to get approximately 7 log CFU/ml (counted by haemocytometer).

Table 6.1 Assam green tea infusion preparation for cooked beef antimicrobial and anti-lipid oxidation investigations

Volume of stock 250 mg/ml tea infusion (ml)	Distilled water (ml)	Tea final concentration (mg/ml)
0	100	0
12.5	87.5	31.3
25	75	62.5
50	50	125
100	0	250

6.2.3 Beef Preparation

The beef was purchased from Phathai market, Chiang Rai and cut into 96 pieces of 6×6×1.5 cm (25 g) and 160 pieces of 6×6×1.5 cm (35 g) for antimicrobial and anti-lipid oxidation activity investigation, respectively. Then, it was cooked by autoclave at 121 °C for 20 min (Alves, 2006)

1. Cooked Beef Preparation for Antimicrobial Activity Investigation

All cooked beef samples (96 pieces of 25 g cooked beef = 2400 g) were inoculated with 24 ml of 7 log CFU/ml stock cultures and mixed throughly to obtain the final inoculum size of 5 log CFU/g. Each piece of inoculated cooked beef (25 g) was placed on a sterile petri disc and kept in a laminar flow with the lid opened for 15 min to let the meat surface dry. Then, 1 ml of assam green tea infusion was applied to the inoculated cooked beef and dried with the lid opened in a laminar flow for 30 s. The inoculated cooked beef was then incubated in a walk-in 4 °C cold room and the microbial analysis was determined everyday for 7 days.

2. Cooked Beef Preparation for Anti-lipid Oxidation Activity Investigation

The cooked beef was separately dipped into 100 ml of 0, 31.3, 62.5, 125 and 250 mg/ml assam green tea infusions and 0.02% BHA/BHT for 30 s. Then, the treated cooked beef was incubated in a walk-in 4 °C cold room for 7 days. The oxidative stability of cooked beef was monitored by using color (10 g), peroxide value (PV) (20 g) and thiobarbituric acid reactive substance (TBARS) (10 g) everyday throughout the storage time.

6.2.4 Microbial Analysis

Microbial determination was carried out according to the method of Ahn (2003). Briefly, the sample (25 g) was aseptically mixed with 225 ml of 0.1% sterile peptone water. The mixture was blended for 2 min in a stomacher. Serial dilution was carried out using 0.1% sterile peptone water and 0.1 ml of the appropriate dilution was spread in duplicate onto an MOX agar and XLD agar to determine the population of *L. monocytogenes* and *S. typhimurium*, respectively. Whereas, 1 ml of appropriate dilution was dropped onto petrifilm of *E. coli* coliform and a Staph express count plate to determine the populations of *S. aureus and E. coli,* respectively. All of the samples were incubated at 37 °C for 24 hrs.

6.2.5 Color of the Cooked Beef Determination

The cooked beef (10 g) was subjected to color evaluation by the *L*, a*,* and *b** (lightness, redness, and yellowness) coordinates using the ColorQuest®XT (HunterLab, Associates Inc., Reston, Virginia, USA). Δ *L*,* Δ *a*,* and Δ *b** value were then calculated by the color differentiation of the first day and the evaluated day color throughout the storage time.

6.2.6 Peroxide Value (PV) Determination

PV determination was carried out according to the method of Prasetyo et al. (2008). The cooked beef (20 g) was ground by a blender at high speed for 2 min. The ground sample was put into a 250 ml Erlenmeyer flask. Iso-Propanol (32 ml) was added to the sample and was then homogenized with a blender at high speed for 30 s. Then, hexane (64 ml) was added and the mixture was homogenized again at high speed for another 30 s. After that, the mixture was centrifuged at 5,000 rpm for 15 min at 4 ˚C, and the supernatant (*n*-hexane phase) was collected, evaporation was carried out by rotary evaporator under reduced pressure at 30 °C to remove the solvent. The obtained extracted beef lipid was mixed with 5 ml of acetic acid-chloroform (3:2

v/v) solution and vortexed for 2 to 4 s. Fifty microliters of 30% (w/v) ammonium thiocyanate solution was added to the sample and vortexed for 2 to 4 s. Then, 50 µl of 1% (v/v) ferrous iron solution was added and vortexed for 2 to 4 s. The sample was incubated for 5 min at room temperature. The absorbance at 470 nm were read using UV/Visible spectrophotometer (Lamda 35, Perkin Elmer Life And Analytical Sciences, Inc, USA). This entire procedure was conducted in subdued light. The standard curve of Fe (III) (0-10 µg/ml) were freshly prepared as shown in Figure C.4. The peroxide values were expressed as mEq of peroxides/kg of the sample, which were calculated using the following equation.

$$\text{Peroxide value (mEq of peroxides/kg)} = (As - Ab)/(55.84 \times mo \times \text{m} \times 2) \qquad (6.1)$$

where As = absorbance of sample

Ab = Absorbance of the blank

m = slope of the Fe (III) calibration (Figure C.4)

mo = mass in gram of the sample (20 g)

55.84 = atomic weight of iron

6.2.7 Thiobarbituric Acid Reactive Species (TBARS) Determination

TBARS was used to determine lipid oxidation in the sample (Juntachote et al., 2007). The cooked beef (10 g) was ground by a blender at high speed for 2 min. The ground sample was mixed with 50 ml of 10% tricloroacetic acid (TCA) solution for 30 s at high speed in a blender. The mixture was filtered using a Whatman filter paper No 1. Five milliliters of the filtrate were mixed with 5 mL of 0.02 M thiobarbituric acid (TBA) solution and heated in a 95 °C bath for 20 min. Then, the absorbance was read at 532 nm by UV-Visible spectrofotometer (Lamda 35, Perkin Elmer Life And Analytical Sciences, Inc, USA). The standard curve of malonaldehyde (0-5 nM/ml) was freshly prepared by acidification of TEP (1,1,3,3-tetraethoxypropane) as shown in Figure C.5. TBARS values were calculated as mg malonaldehyde (MAD)/kg sample according to the standard curve (Juntachote et al., 2007).

6.2 Results and Discussion

6.2.1 Antimicrobial Activity of Tea Infusion in Cooked Beef

The survival growth of *S. aureus* in cooked beef containing green tea infusion is shown in Figure 6.1 over 7 days. It was found that tea of 250 mg/ml, *A* and *B* reduced *S. aureus* population to an undetectable level in the first day of storage, while less inhibition was observed on cooked beef at 125 mg/ml concentration *A* and *B*, indicated by 2 days was needed for bacterial inactivation. Whereas, cooked beef containing 0.02% BHA/BHT needed 3 days to inactivate *S. aureus* to an undetectable level from the initial inoculum size of 5 log CFU/g sample. These suggest that higher concentration produces a higher inhibitory effect.

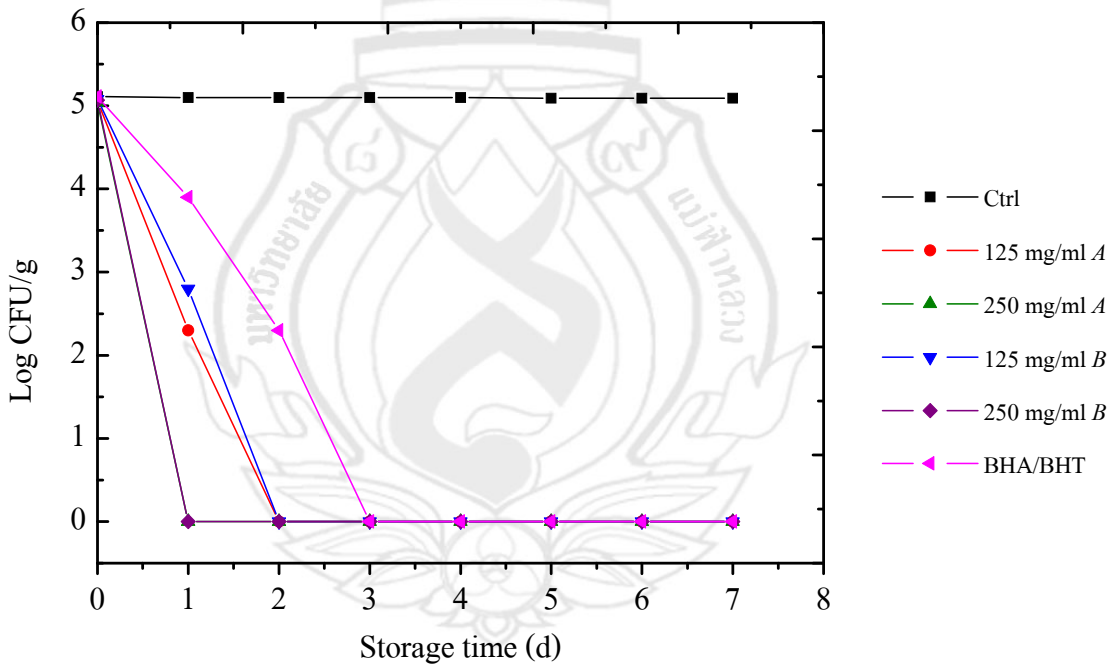


Figure 6.1 Population of *S. aureus* in the cooked beef incubated at 4 °C for 7 days

Figure 6.2 shows the survival of *L. monocytogenes* in cooked beef containing assam green tea infusion incubated at 4 °C for 7 days. It was found that *L. monocytogenes* was completely reduced by 250 mg/ml of *A* and *B* by 3 days storage, whereas 4 days were needed to inhibit *L. monocytogenes* by other concentrations of *A* and *B* and by BHA/BHT, respectively.

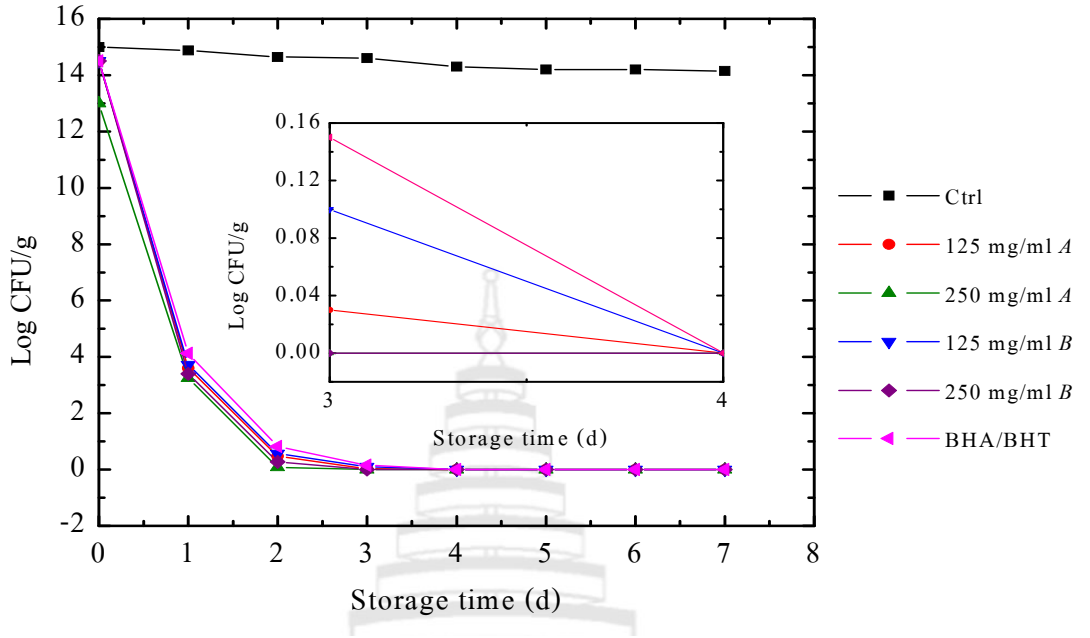


Figure 6.2 Population of *L. monocytogenes* in the cooked beef incubated at 4 °C for 7 days (inset on day 3 and 4)

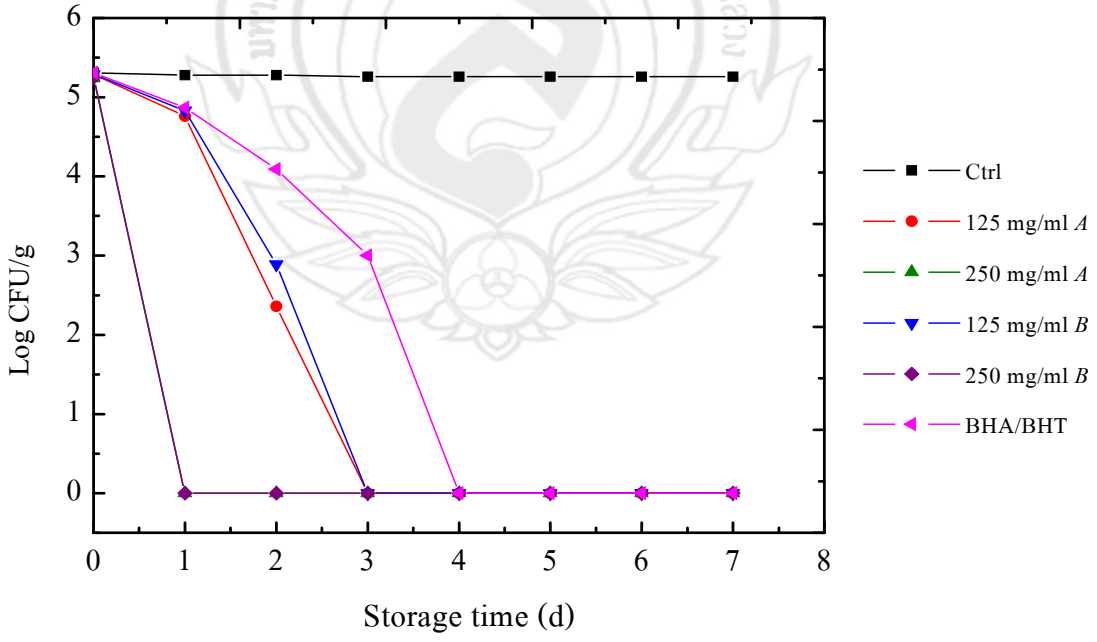


Figure 6.2 Population of *S. typhimurium* in the cooked beef incubated at 4 °C for 7 days

The survival of *S. typhimurium* in cooked beef containing assam green tea infusion incubated at 4 °C for 7 days is shown in Figure 6.3. It was found that all assam green tea infusion concentrations needed 3 days to inactivate the *S. typhimurium* except tea infusion at the concentration of 250 mg/ml of *A,* which took only 1 day to reduce *S. typhimurium* to an undetectable level. It took 4 days for 0.02% BHA/BHT to completely inhibit *S. typhimurium.* The less inhibitory effect was also observed in *E. coli,* requiring 3 days to inactivate these bacteria to an undetectable level from the initial inocolum size of 5 log CFU/g as shown in Figure 6.4.

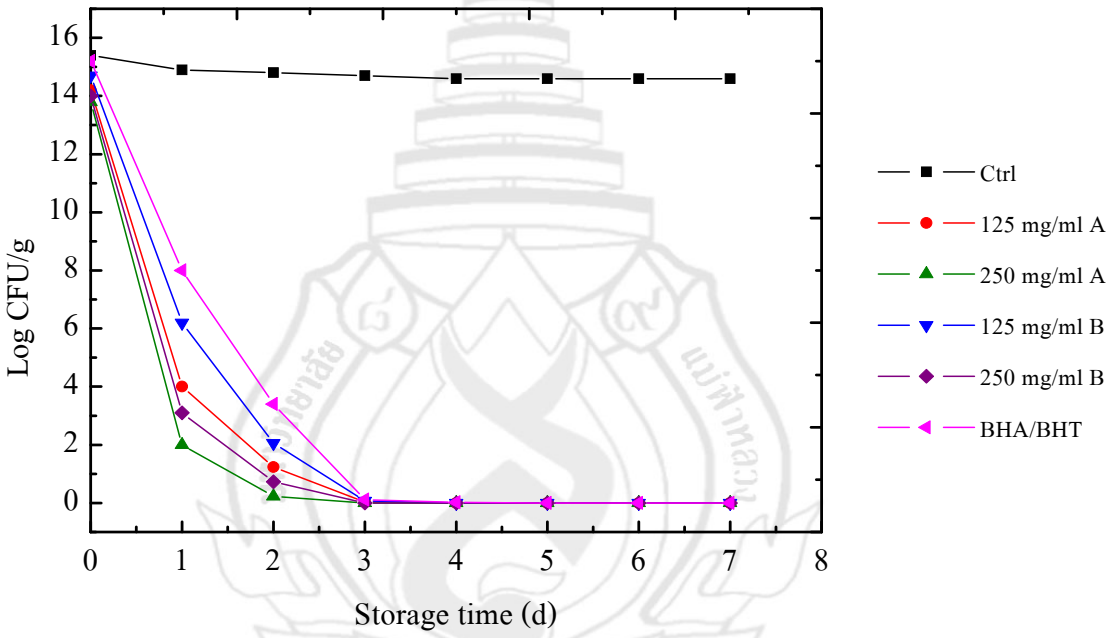


Figure 6.4 Population of *E. coli* in the cooked beef incubated at 4 °C for 7 days

It was also oberved that all tested bacteria still survive throughout the storage time without any tea infusion and 0.02 % BHA/BHT addition. The results illustrated that the gram positive is more sensitive than gram negative. These results were consistent with the results obtained in Chapter 3 to 5.

6.2.2 Anti-lipid Oxidation Activity of Tea Infusion in Cooked Beef

The anti-lipid oxidation activity of assam green tea infusions in cooked beef was investigated by means of PV and TBARS, over 7 days of refrigerated storage. PV refers to the total concentration of peroxides and hydroperoxides obtained as a result of an oxidative reaction. TBARS is the determination of secondary oxidation products, such as aldehydes, ketones, hydrocarbons and alcohols after the oxidative reaction. The higher peroxide value and TBARS indicate the increase of lipid peroxides products obtained as a result of rancidity in cooked beef. PV and TBARS in cooked beef are shown in Figure 6.5 and 6.6, respectively. PV in all samples of cooked beef increased throughout the storage time as shown in Figure 6.5. However, the PV rate of increase in cooked beef treated with assam green tea infusion was significantly lower than those of 0.02% BHA/BHT treated beef and the control, as indicated by the lower slope. These suggest that lipid oxidation was effectively retarded by *A* and *B* than BHA/BHT. *A* and *B* at the higher concentration (250 mg/ml) exhibited higher anti-lipid oxidation activity on the cooked beef throughout the storage time.

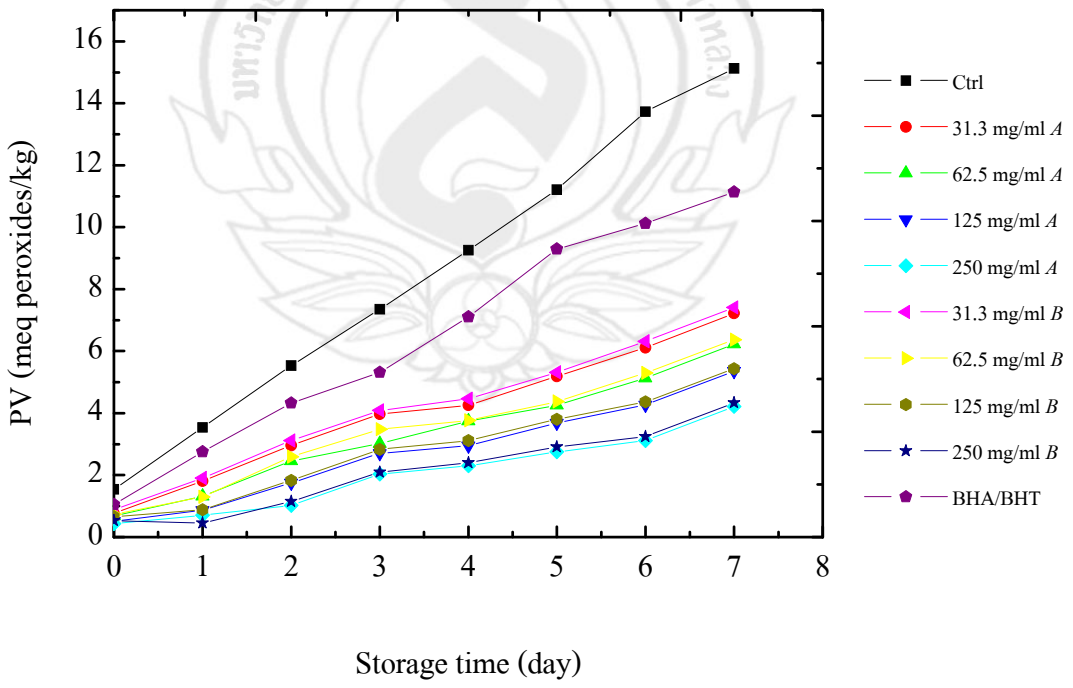


Figure 6.5 PV (meq peroxide/kg) in the cooked beef containing different concentrations of assam green tea infusions and 0.02 % BHA/BHT over 7 days of storage at 4 °C

TBARS values in all samples of cooked beef also increased during storage at 4 °C. However, the TBARS value rate of increase in cooked beef treated with assam green tea infusion was significantly lower than those of BHA/BHT treated and the control as shown in Figure 6.6 (inset). These indicate that lipid oxidation was effectively retarded by *A* and *B* than BHA/BHT. *A* and *B* at the highest concentration (250 mg/ml) exhibited the most antioxidative activity on the cooked beef throughout the storage. The antioxidant activity of assam green tea infusion significantly increased with the tea infusion concentration ($p \leq 0.01$).

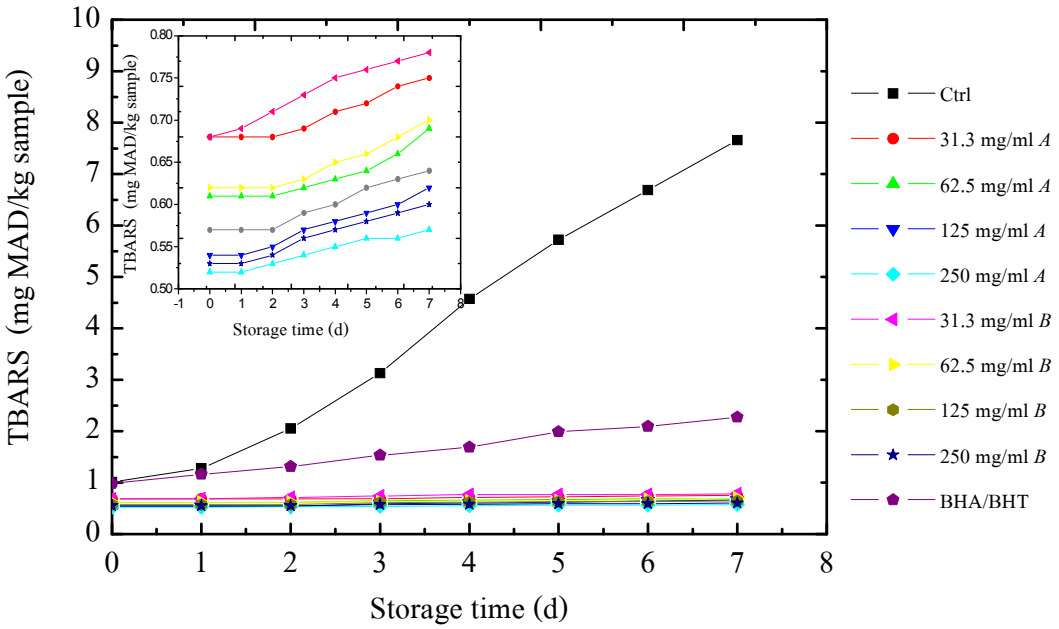


Figure 6.6 TBARS (mg MAD/kg) in the cooked beef containing different concentrations of assam green tea infusions and 0.02% BHA/BHT over 7 days of storage at 4 °C (inset) enlarged TBARS of the cooked beef containing different tea concentrations

The results are consistent with Tang et al. (2001) who reported that the addition of tea catechins at the concentration of 300 mg/kg sample in cooked beef resulted in a significant (p<0.05) reduction in the formation of TBARS as compared to BHT, BHA, TBHQ and vitamin E. The high anti-lipid oxidation activity of green tea were responsible by its high polyphenols

content, which was observed in Chapter 3. It may play an important role in autooxidation termination of cooked beef by functioning as free radical scavengers (Ahn, 2003).

The treatment effects on color changes of cooked beef during the refrigerated storage are shown in Figure 6.7 to 6.9. As shown in Figure 6.7, the lightness (ΔL^* values) significantly increased in assam green tea infusion compared to 0.02% BHA/BHT. After 4 days of storage, tea infusion tended to decrease. Among the tea infusion, *A* at the concentration of 250 mg/ml had the highest curve of lightness ($p \leq 0.05$), indicating that *A* had the greatest effect on lightness change in the cooked beef than that of *B* and BHA/BHT.

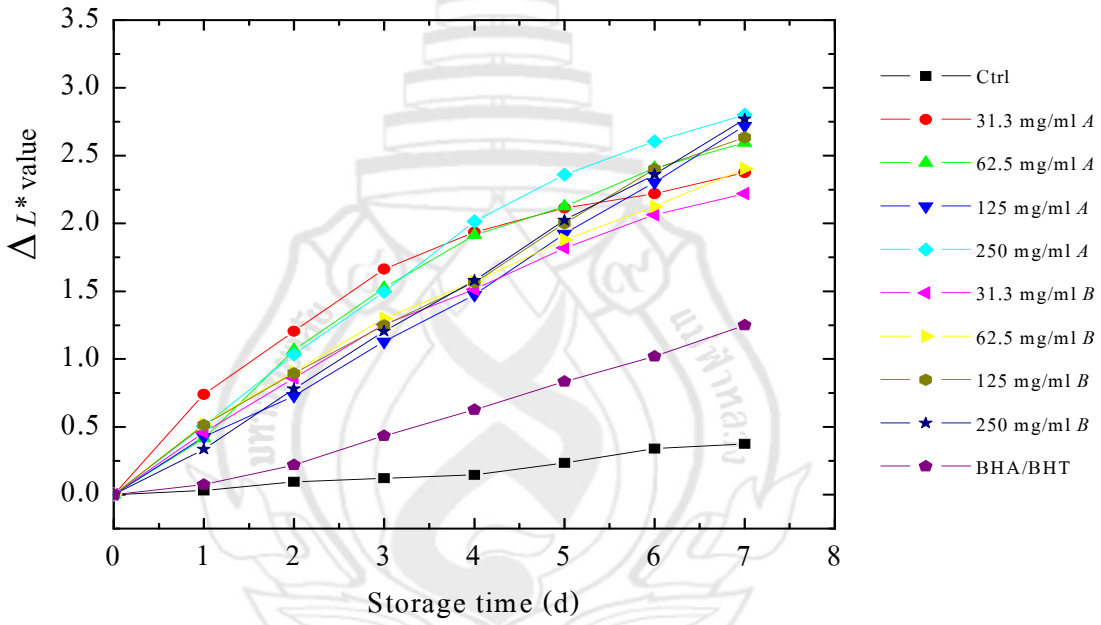


Figure 6.7 ΔL^* value in the cooked beef containing different concentration of assam green tea infusions and 0.02% BHA/BHT over 7 days of storage at 4 °C

The addition of *A* significantly decreased Δa^* value ($p \leq 0.05$) during the storage time. *A* at the highest concentration (250 mg/ml) exhibited the highest Δa^* values in the cooked beef, indicating that *A* can retard the reduction of redness in the cooked beef. On day 7, the cooked beef containing assam green tea infusion in all concentration were more red than the other treatments. Significant differences of Δb^* values were observed in the control and assam green

tea infusion treated cooked beef during the refrigerated storage. However, *A* and *B* considerably lowered the Δ *b** values in the cooked beef. These indicate that color measurement was more effectively retarded by *A* and *B* than by BHA/BHT.

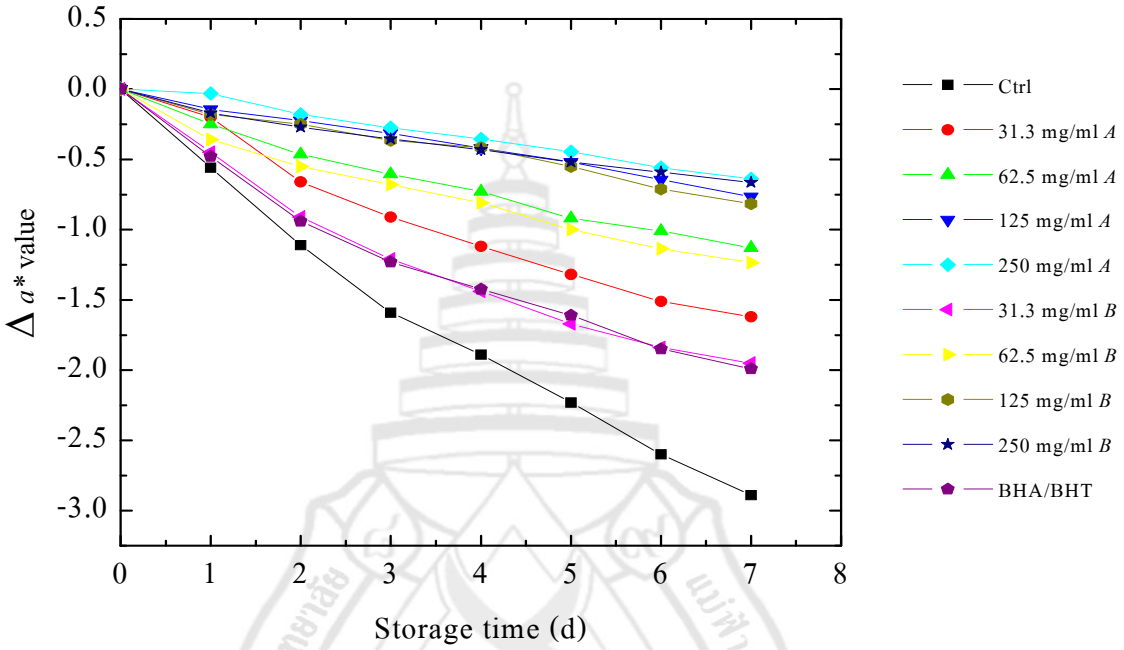


Figure 6.8 Δ *a** value in the cooked beef containing different concentration of assam green tea infusions and 0.02% BHA/BHT over 7 days of storage at 4 °C

The retention of color change in cooked beef containing assam green tea infusion may result from their antioxidative effects and their contribution of pigments. With high polyphenol content, assam green tea infusion can retard the free radical chain reaction in the oxidation process (Cuppett, 2001). The results are similar to the results obtained by Mitsumoto et al. (2003) who reported that green tea extract increased *L** value and decreased the *a** and *b** value. As the high quality meat, which have more lightness, rednees but less yellowness (Ahn et al., 2006), assam green tea infusion showed potent to retard the color change in cooked beef and produce better quality cooked beef compared to the beef treated with BHA/BHT.

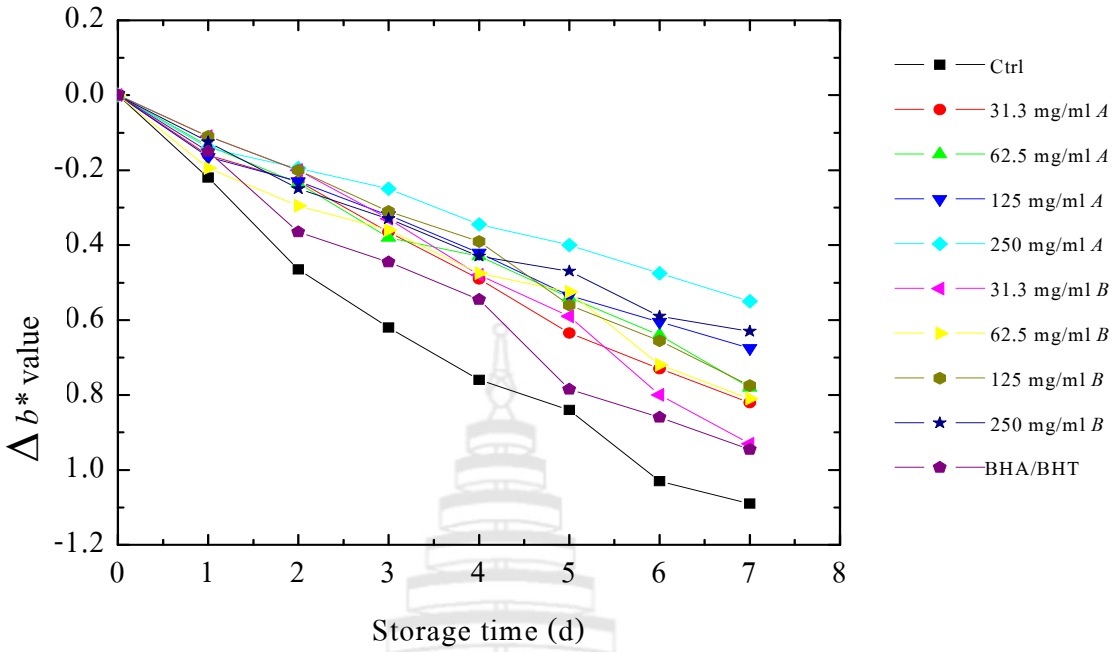


Figure 6.9 Δ *b** value in the cooked beef containing different concentration of assam green tea infusions and 0.02% BHA/BHT over 7 days of storage at 4 °C

6.3 Conclusion

The two commercial assam green teas, *A* and *B* are significantly effective at reducing the number of *S. aureus, L. monocytogenes, S. typhimurium* and *E. coli*, retarded lipid oxidation and color degradation. These suggest that the assam green tea infusion might be a potential natural preservative used to extend shelf life by improving the microbial safety, possibly showing the same effect to oxidative and color stability of cooked meat and other meat products.

CHAPTER 7

CONCLUSION AND RECOMMENDATION

7.1 Conclusion

There were differences among the commercial assam green tea available in Chiang Rai in terms of antioxidant and antimicrobial activities particularly at high concentration. The investigation of those activities showed that *A* exhibited the most antioxidant and antimicrobial activities, followed by *B, C, D* and *E,* respectively. These are indicated by the highest TPC and antioxidant activity of assam green tea *A* and *B*. There are two main catechins presented in commercial assam green tea, EC and EGC. The tea with high TPC, antioxidant and antimicrobial activities contained EC and EGC. This suggests that antioxidant and antimicrobial activities are contributed by these 2 compounds. In addition, gram positive bacteria was found to be more sensitive than gram negative bacteria.

Similar results were observed when the tea infusions was applied to a liquid medium and watermelon juice. The application of those infusions in watermelon juice found to significantly reduce the pH of watermelon juice. *A* and *B* could completely inactivate *S. aureus, L. monocytogenes, S. typhimurium* and *E. coli* within 2, 3, 5 and 6 days, respectively. The inhibitory effect of those tea infusions were then applied in cooked beef. It was found that *A* and *B* infusion at the highest concentration (250 mg/ml), could effectively reduce the population of *S. aureus, L. monocytogenes, S. typhimurium* and *E. coli* to an undetectable level within 2 days of storage. *A* and *B* also showed high anti-lipid oxidation activity compared to BHA/BHT and control. The addition of those tea infusion in cooked beef significantly retained the lightness, redness but retarded yellowness formation in cooked beef.

This present study observed that compared to the common antioxidant and antimicrobials used, BHA/BHT, assam green tea infusion showed more effects to the reduction of lipid oxidation, discoloration and microbial growth in both liquid and solid food models. These results suggest that green tea infusion provided a significant improvement in terms of the microbial safety and quality of watermelon juice and cooked beef and it might be a good alternative for preserving other types of food with the same purposes.

7.2 Recommendation

7.2.1 Color determination in this experiment was only conducted in one point of the cooked beef. In order to get more accuracy, the determination in many points of cooked beef is needed for further study.

7.2.2 Different foods have different components and different antioxidants and antimicrobial susceptibilities. Further study should be conducted in other food model systems.

7.2.3 In the present study, the assam green tea infusion used possesed antioxidant and antimicrobial properties *in vitro* as well as increased the shelf life of foods. At varied concentrations, assam green tea infusion showed different activities of both, antioxidant and antimicrobial activities. However, the use of high concentrations of assam green tea could result in adverse effects on the organoleptic properties of food products. Therefore, further studies are needed to determine the effective concentrations of these compounds to the sensorial threshold and the acceptable level of assam green tea that would achieve antimicrobial and antioxidant activities in food products without adversely affecting the organoleptic properties.

7.2.4 Further study about the combination of assam green tea infusion with other preservatives such as high-intensity pulsed electric fields (HIPEC) and high hydrostatic pressure is needed to enhance the antioxidant and antimicrobial activity in food model systems.

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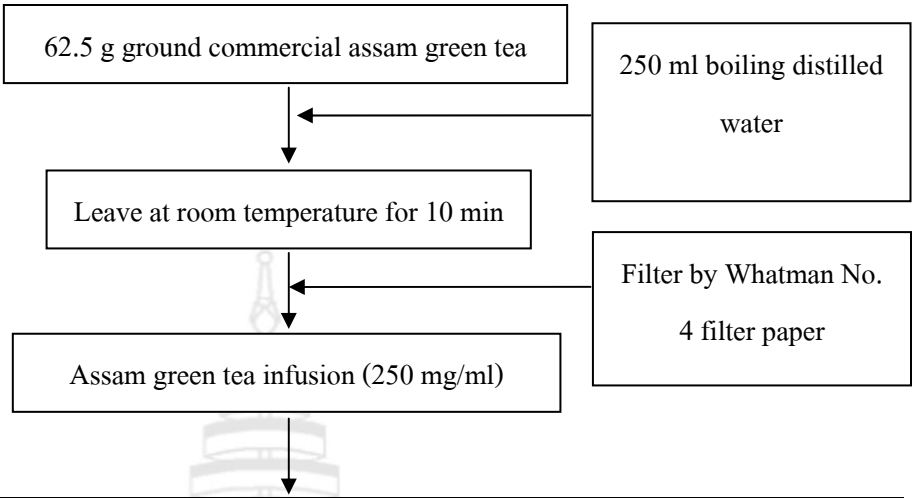
APPENDICES

APPENDIX A

EXPERIMENTAL SET UP

A.1 Assam green tea preparation



Volume of stock 250 mg/ml tea solution (ml)	Distilled water (ml)	Tea final concentration (mg/ml)
0	20	0
2	18	25
4	16	50
6	14	75
8	12	100
10	10	125
12	8	150
14	6	175
16	4	200
18	2	225
20	0	250

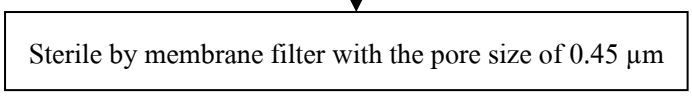


Figure A.1 Assam green tea infusion preparation

A.2 Total polyphenol content

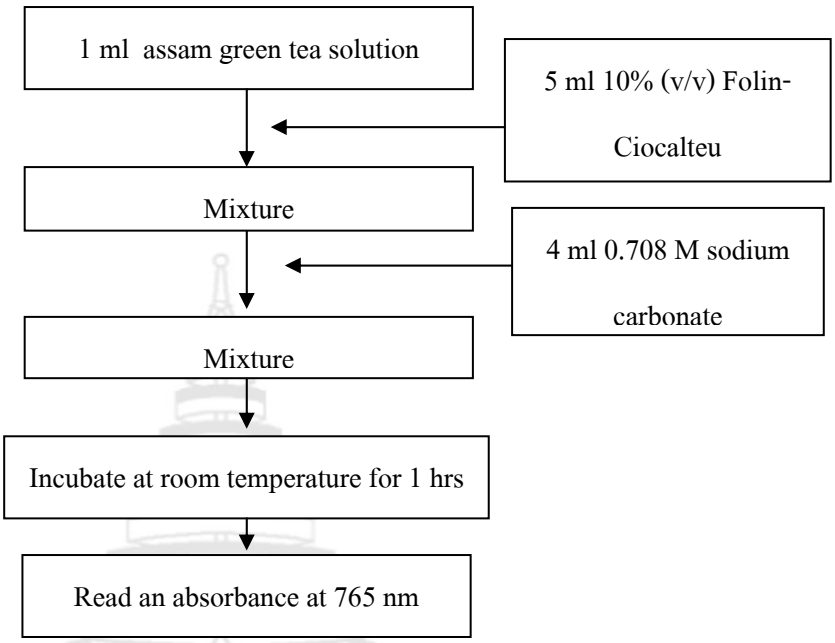


Figure A.2 Determination of total polyphenol content

A.3 DPPH scavenging activity

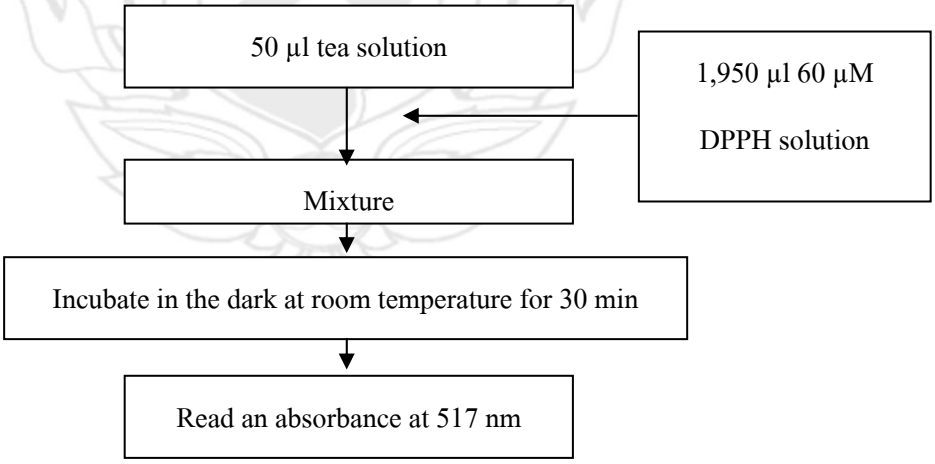


Figure A.3 Determination of antioxidant activity

A.4 Inhibitory Concentration of 50% DPPH radical (IC_{50})

Various concentrations of the assam green tea infusion were prepared according to the Table A.4. Then, the DPPH scavenging activity of the assam green tea infusion was determined and % inhibition was calculated. The tea concentration was plotted against the % inhibition. The concentration of assam green tea infusion that can inhibit 50% DPPH radical formation was then defined as the inhibitory cocentration of 50% DPPH radical (IC_{50}) as shown in Figure A.4.

Table A.4 Assam green tea infusion preparation for IC_{50} investigation

Volume of stock 250 mg/ml tea solution (ml)	Distilled water (ml)	Tea Final concentration (mg/ml)
0	20	0
46.87	3.13	15.63
43.75	6.25	31.25
37.5	12.5	62.5
25	25	125
0	50	250

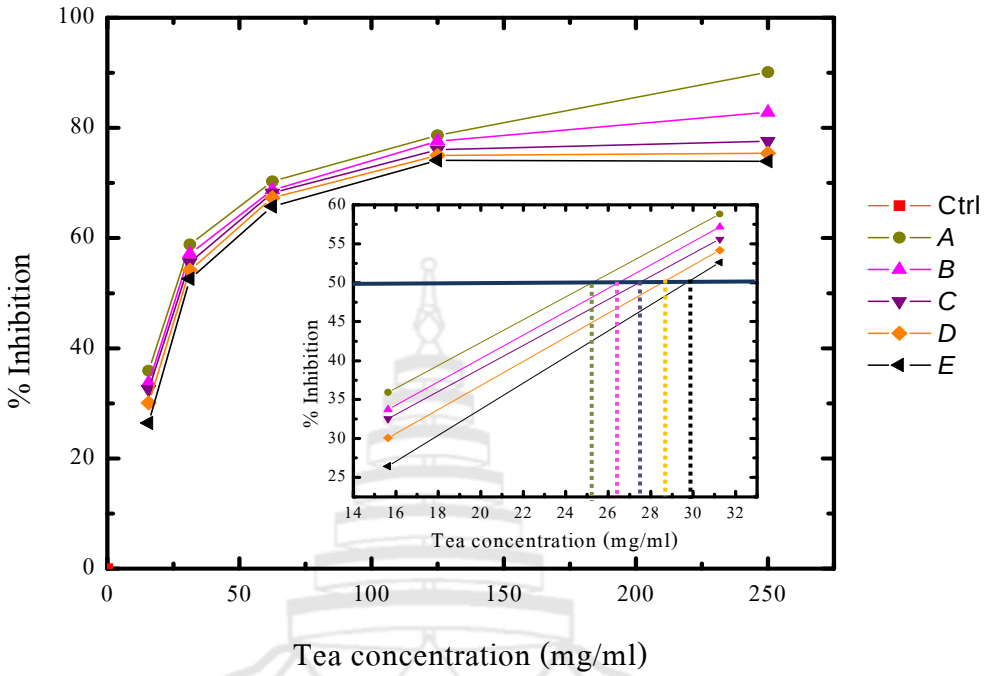


Figure A.4 IC_{50} of assam green tea infusion

A.5 Antimicrobial activity of commercial assam green tea infusion in solid medium by agar diffusion method

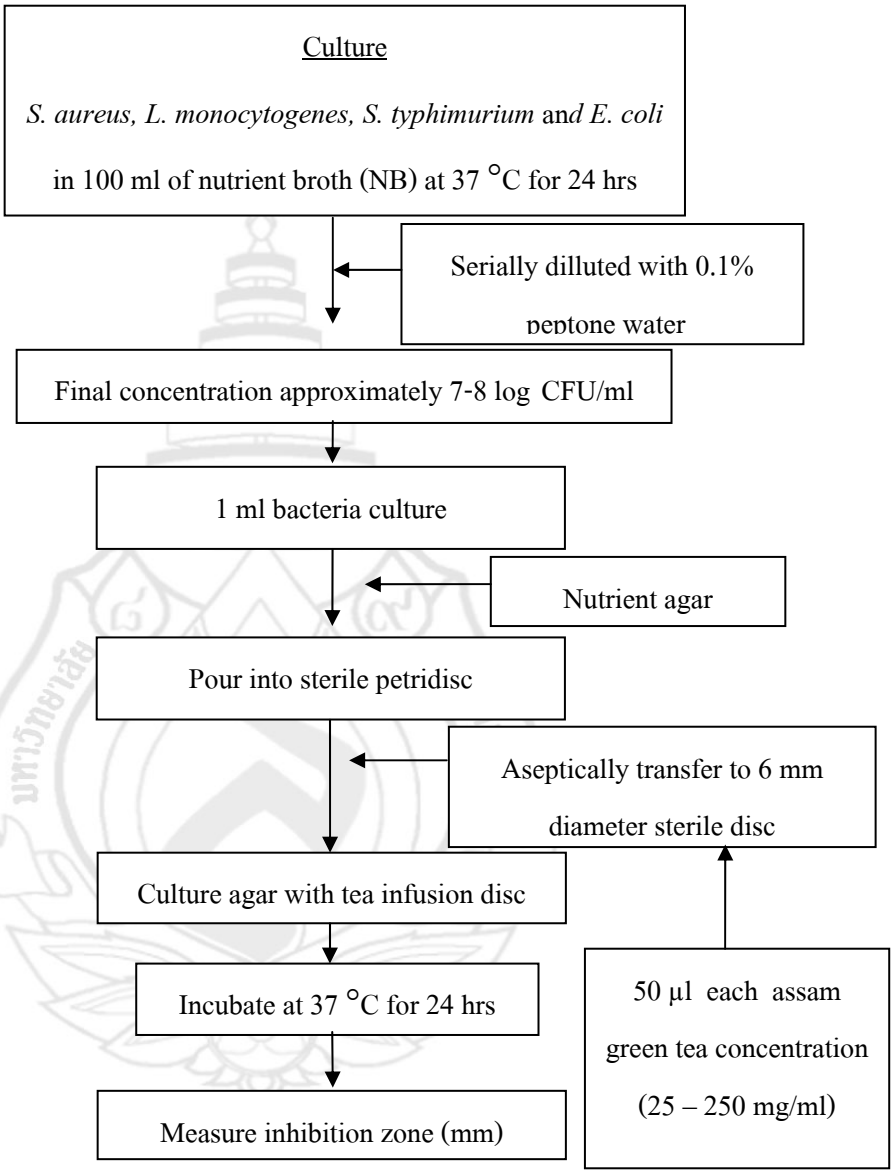
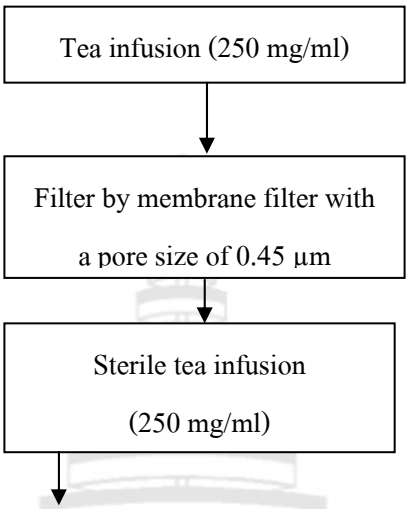


Figure A.5 Determination of antimicrobial activity of assam green tea infusion in solid medium by agar diffusion method

A.6 Antimicrobial activity of commercial assam green tea in liquid medium by macrodillution method



BHI (g)	Volume of stock 250 mg/ml tea infusion (ml)	Distilled water (ml)	BHI-tea mixture final concentration (mg/ml)
1.48	0	20	0
1.48	2	18	25
1.48	4	16	50
1.48	6	14	75
1.48	8	12	100
1.48	10	10	125
1.48	12	8	150
1.48	14	6	175
1.48	16	4	200
1.48	18	2	225
1.48	20	0	250

Figure 6.1 BHI-tea mixture preparation

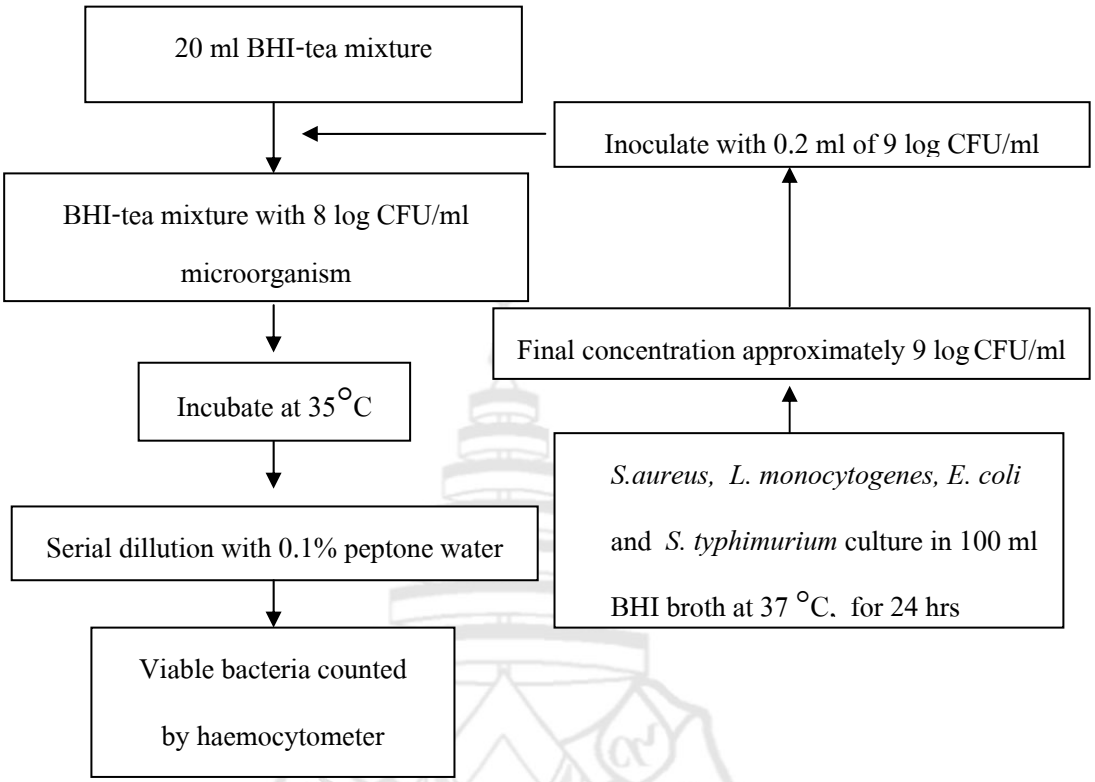
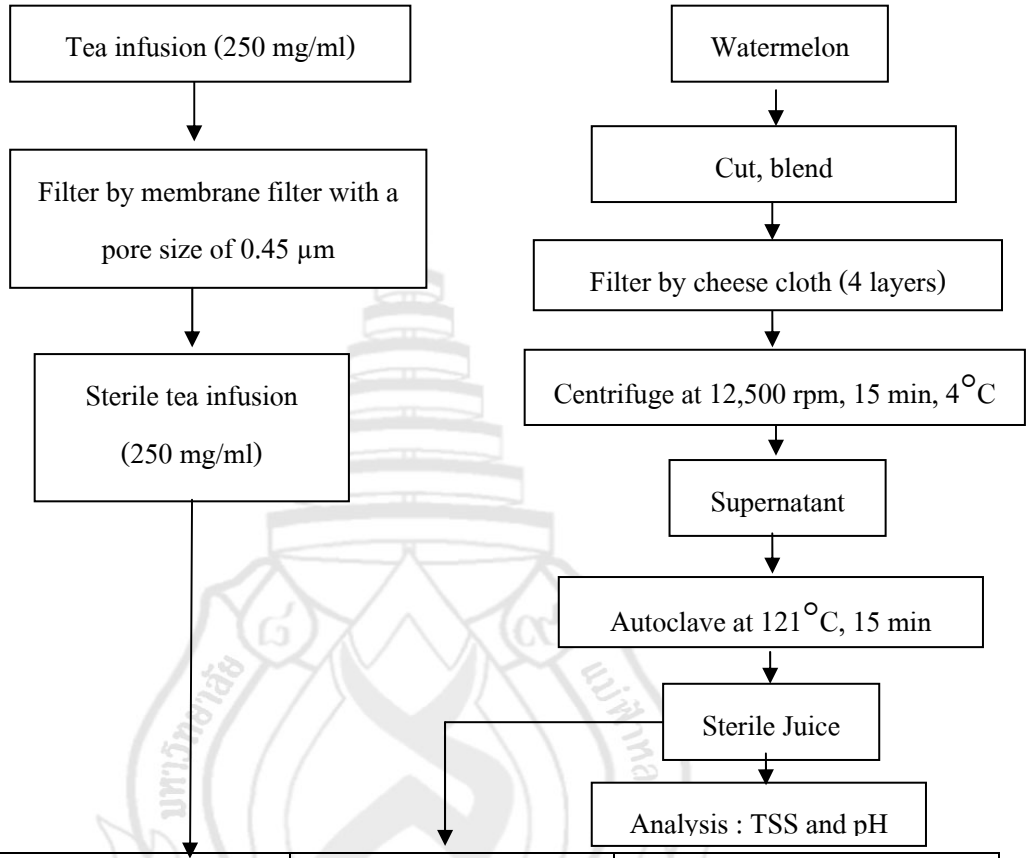


Figure A.6.2 Determination of antimicrobial activity of commercial assam green tea infusion in liquid medium by macrodillution method

A.7 Antimicrobial activity of commercial assam green tea infusion in watermelon juice



Stock 250 mg/ml tea infusion (ml)	Sterile juice (ml)	Tea final concentration in watermelon juice (mg/ml)
20	0	250
14	6	175
8	12	100
6	14	75
0	20	ctrl

Figure A.7.1 Watermelon juice-tea mixture preparation

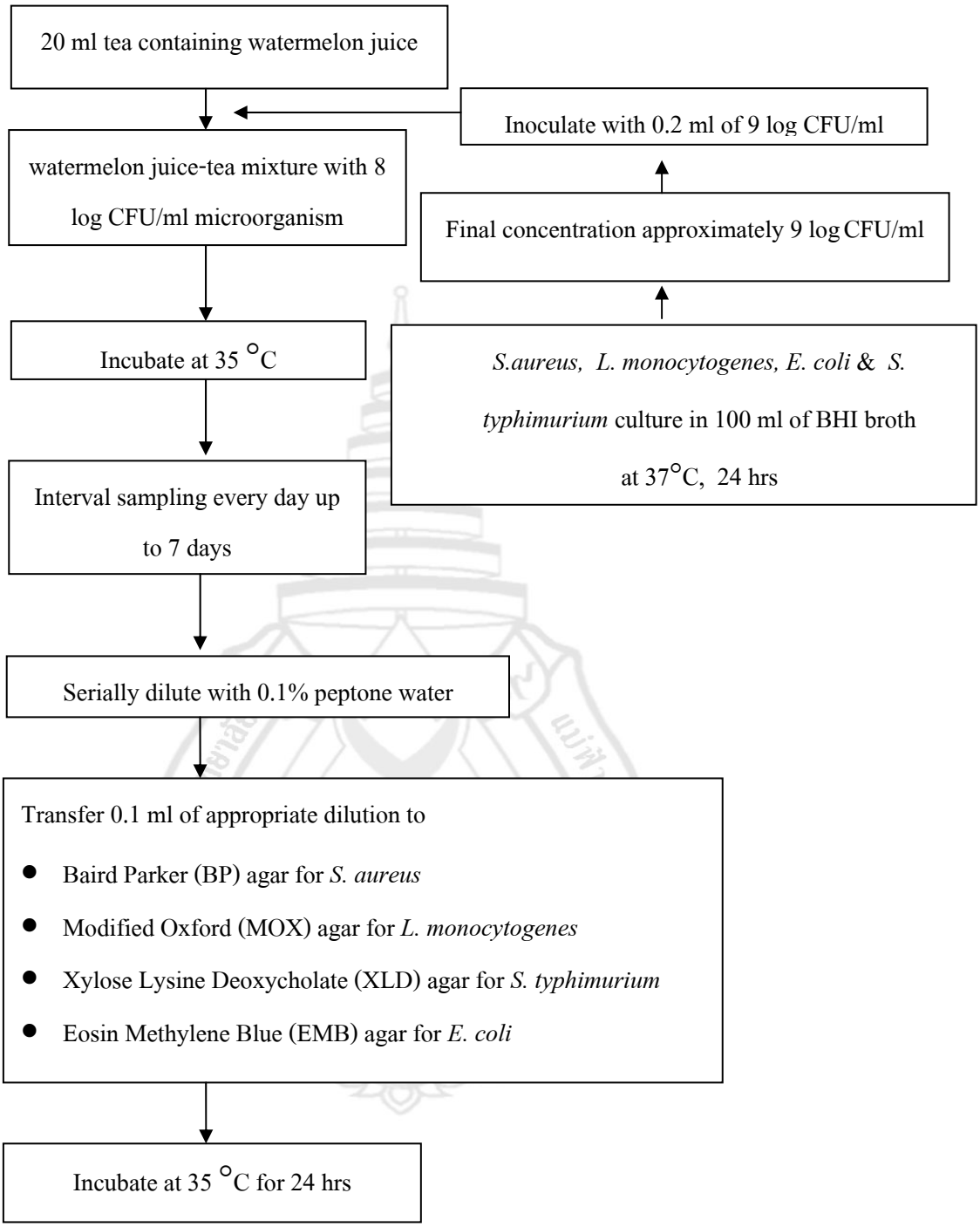


Figure A.7.2 Determination of antimicrobial activity of commercial assam green tea infusion in watermelon juice

A.8 Antimicrobial activity of commercial assam green tea infusion in cooked beef

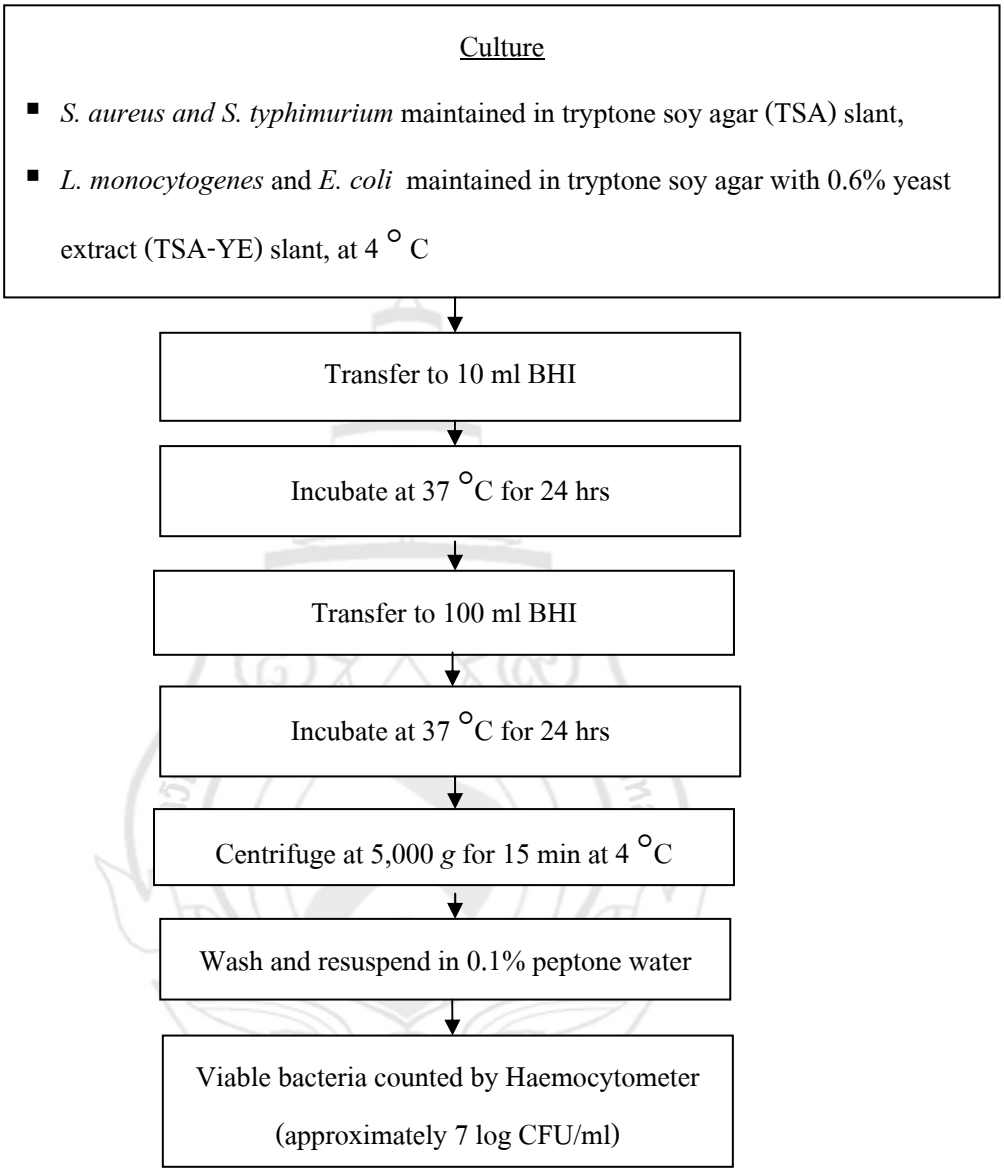


Figure A.8.1 Microorganism preparation for antimicrobial activity of commercial assam green tea infusion in cooked beef investigation

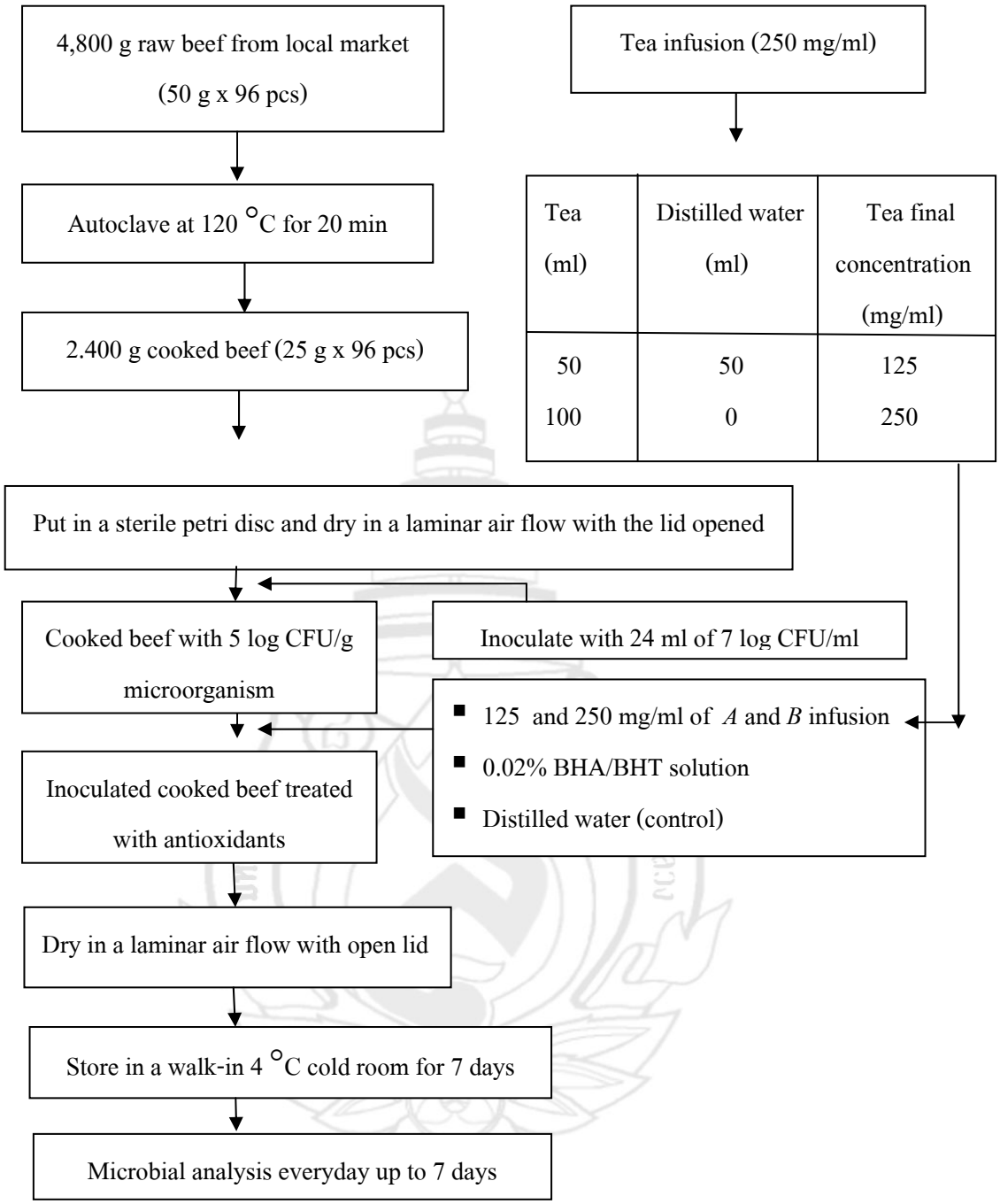


Figure A.8.2 Beef preparation for antimicrobial activity of commercial assam green tea infusion in cooked beef investigation

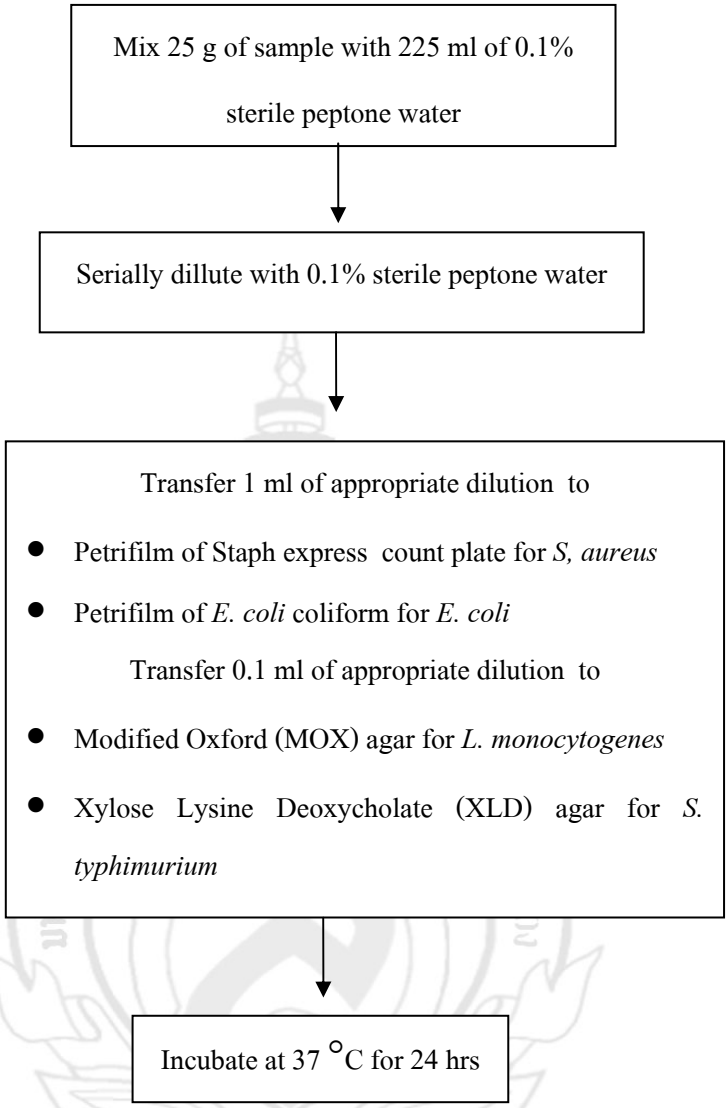


Figure A.8.3 Determination of antimicrobial activity of commercial assam green tea infusion in cooked beef

A.9 Anti-lipid oxidation activity of commercial assam green tea infusion in cooked beef

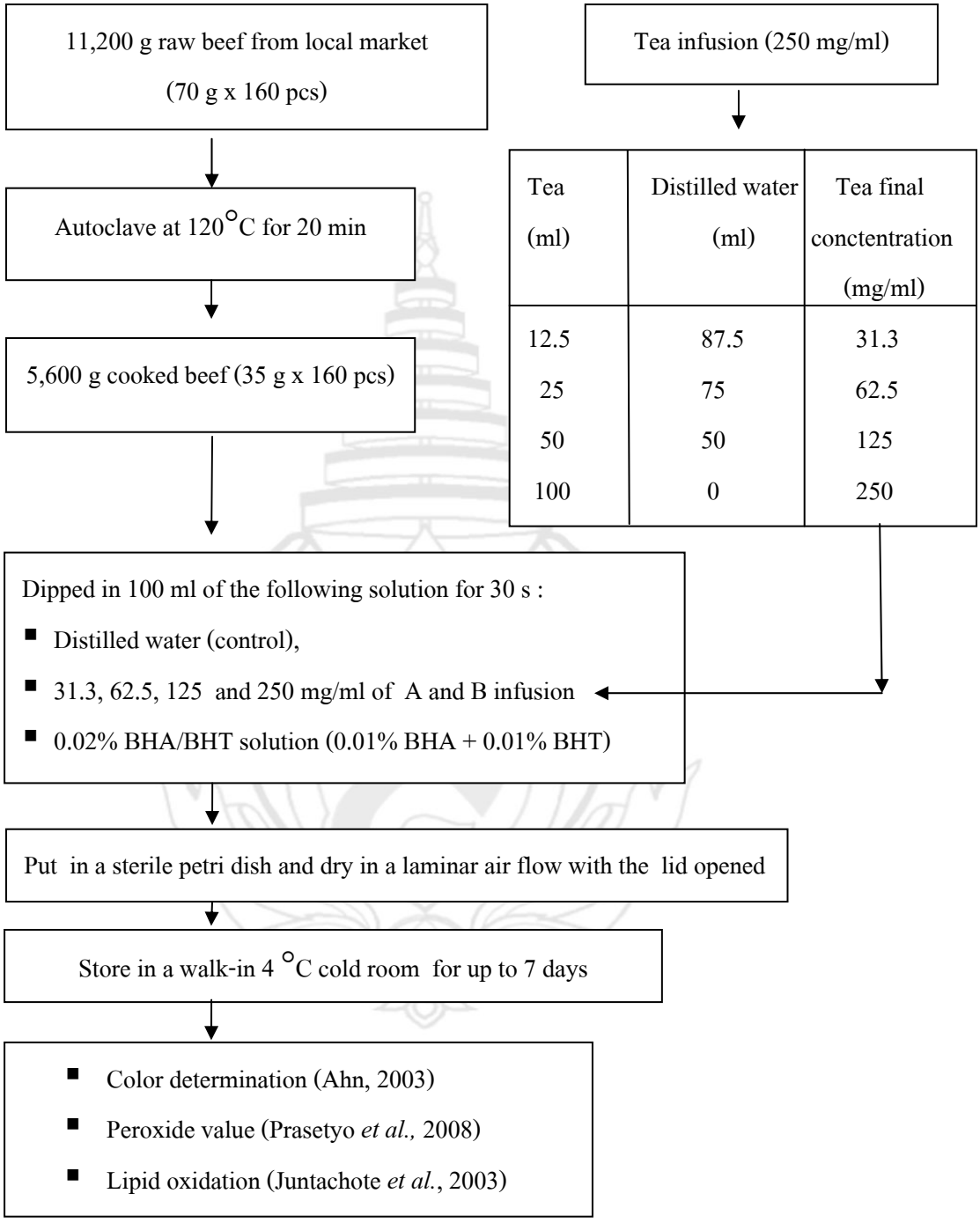


Figure A.9.1 Beef preparation for anti-lipid oxidation activity of commercial assam green tea infusion investigation

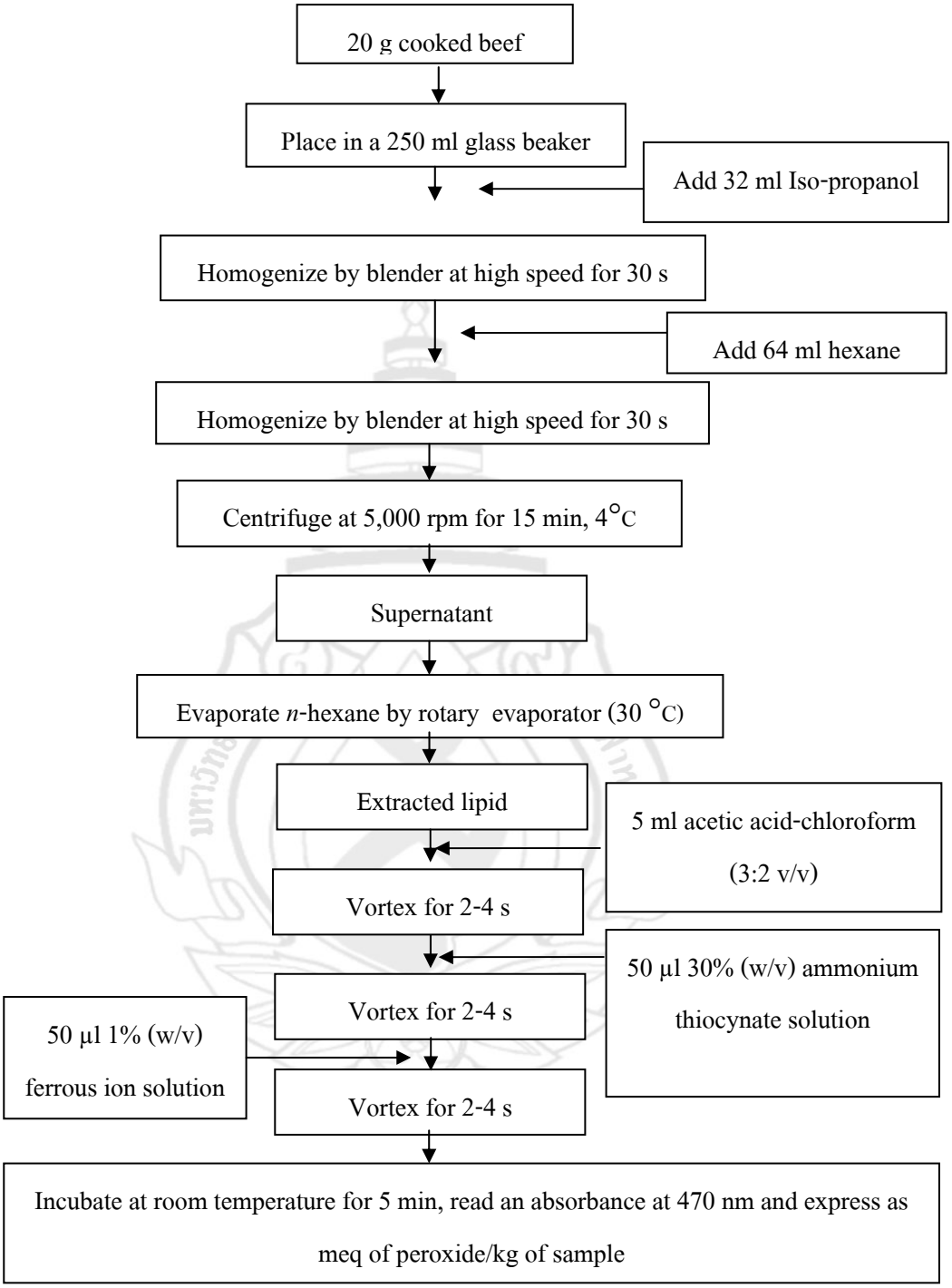


Figure A.9.2 Determination of peroxide value of commercial assam green tea infusion in cooked beef

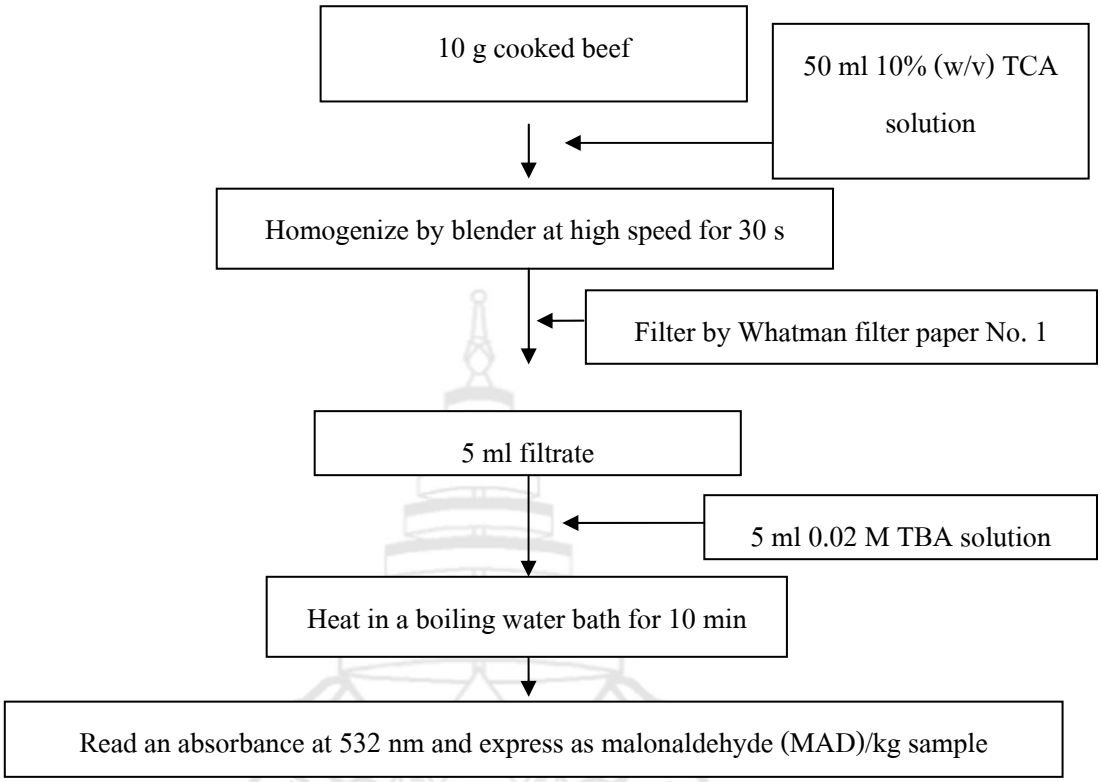


Figure A.9.3 Determination of TBARS of commercial assam green tea infusion in cooked beef

APPENDIX B

MICROBIAL GROWTH CURVE

B1. Growth curve of *S. aureus*

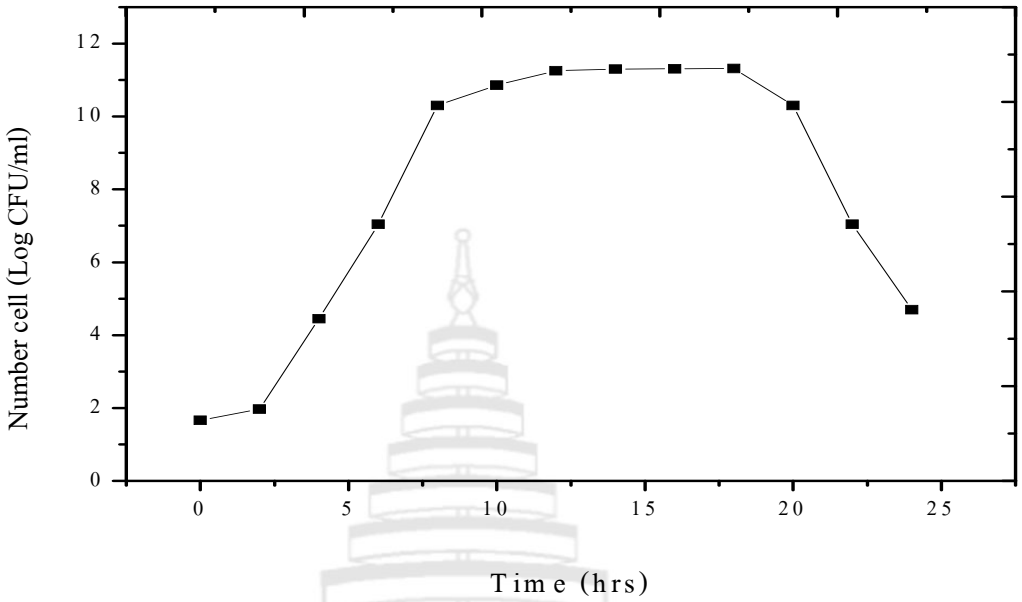


Figure B.1 Growth curve of *S. aureus*

B2. Growth curve of *L. monocytogenes*

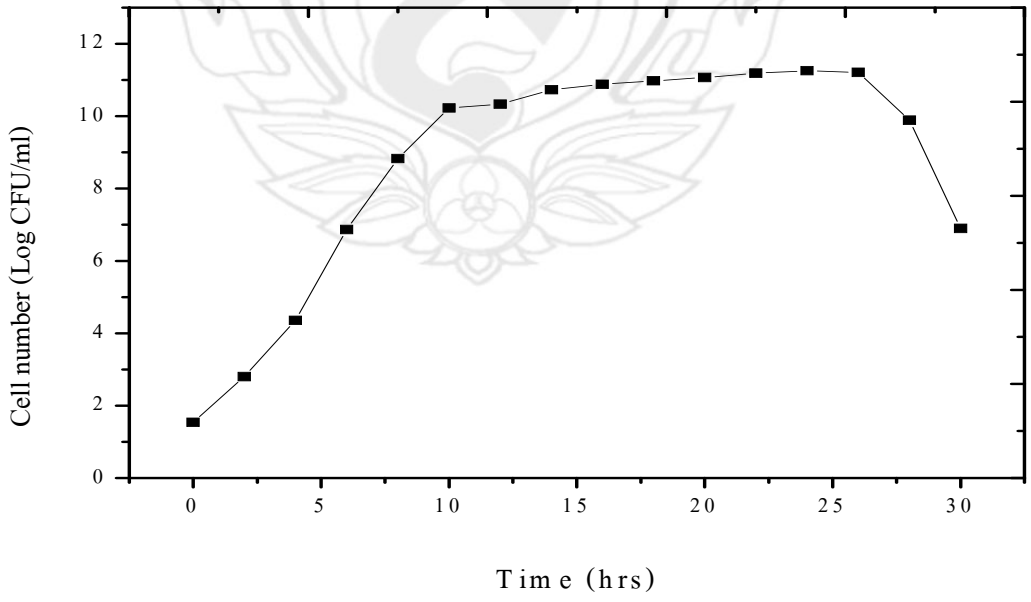


Figure B.2 Growth curve of *L. monocytogenes*

B3. Growth curve of *S. typhimurium*

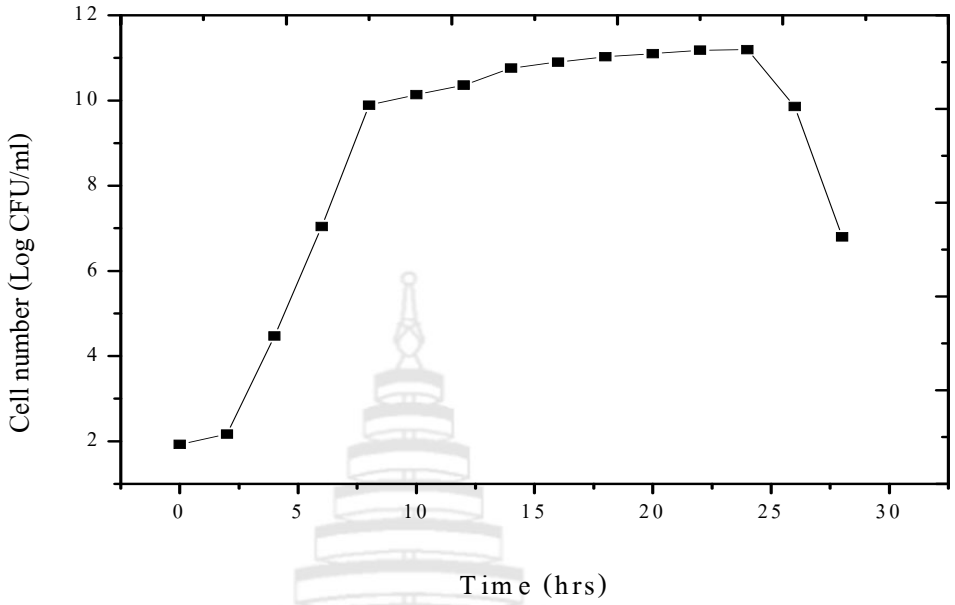


Figure B.3 Growth curve of *S. typhimurium*

B4. Growth curve of *E. coli*

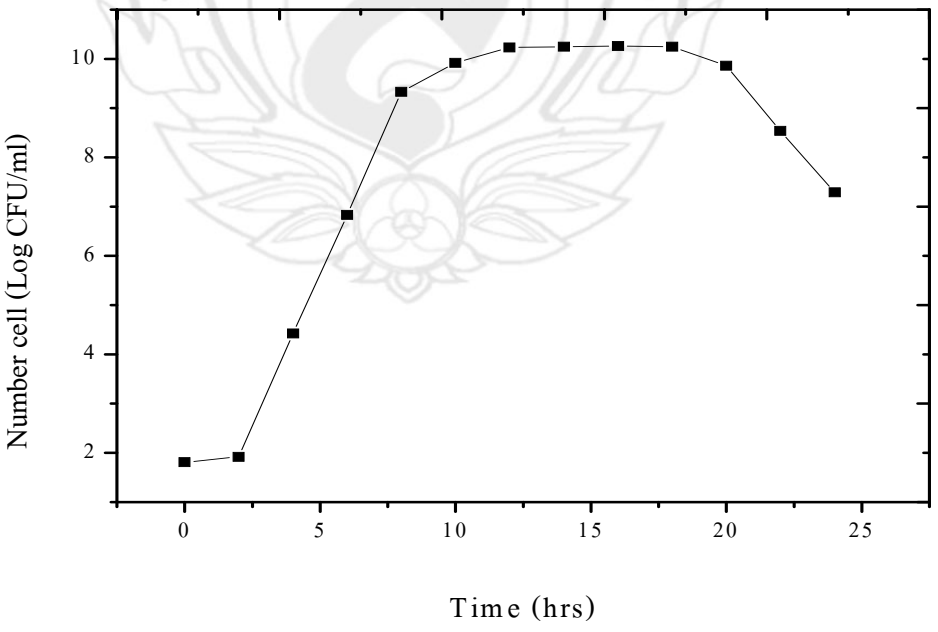


Figure B.4 Growth curve of *E. coli*

APPENDIX C

STANDARD CURVE

C 1. Standard curve of total polyphenol content

Total polyphenol was determined according to the method of ISO:14502-1 (2005). Briefly, 0.1 g of gallic acid was dissolved with 100 ml of distilled water to get the stock standard gallic acid of 1000 µg/ml. Then, 1, 2, 3, 4 and 5 ml of the stock standard were mixed with 99, 98, 97, 96, and 95 ml of distilled water in order to get 10, 20, 30, 40 and 50 µg/ml gallic acid. Each concentration (1 ml) was throughly mixed with 5 ml of 10% (v/v) Folin-Ciocalteu reagent in a test tube. After 30 min, 4 ml of 0.708 M sodium carbonate was added to the test tube and then the mixture was allowed to stand at room temperature for 1 h. The optical density of the obtained solution was determined by spectrophotometer (Lamda 35, Perkin Elmer Life and Analytical Sciences, Inc, USA) at a wavelength of 765 nm and the standard curve was plotted as shown in Figure C.1.

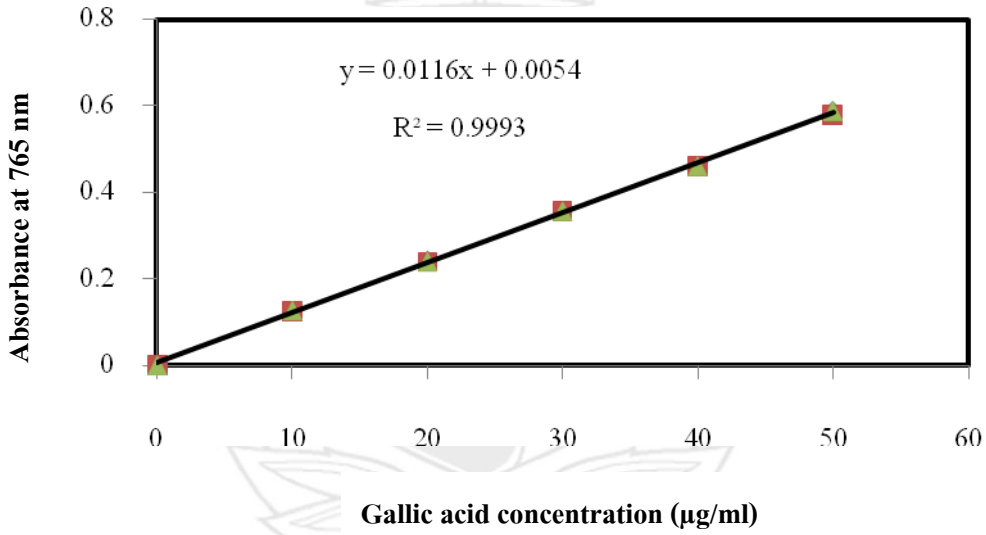


Figure C.1 Standard curve of total polyphenol content as gallic acid (µg/ml)

C.2 Standard curve of antioxidant activity

Antioxidant activity was determined according to the method of Songklanakarin (2004). Briefly, 0.0250 g of trolox was dissolved with 10 ml of absolute methanol to get the stock standard trolox of 10 µM. Then, 0.2, 0.4, 0.6, 0.8, and 1 ml of stock standard solution were

mixed with 9.8, 9.6, 9.4, 9.2 and 9.0 ml of absolute methanol in order to get 200, 400, 600, 800 and 1000 µM of trolox solution. Fifty microliters of each concentration was throughly mixed with 1,950 µl of 60 µM DPPH solution. The mixture was allowed to stand at room temperature for 30 min in the dark. The optical density of the obtained solution was determined by spectrophotometer (Lamda 35, Perkin Elmer Life and Analytical Sciences, Inc, USA) at the wavelength of 517 nm. The standard curve was then plotted as shown in Figure C.2.1 and C.2.2. The inhibition percentage was then calculated by the following equation.

$$\% \text{ inhibition} = \frac{Abs_{ctrl} - Ab_{std}}{Abs_{ctrl}} \times 100$$

where $Abs_{control}$ = absorbance of the control

$Abs_{standard}$ = absorbance of the standard

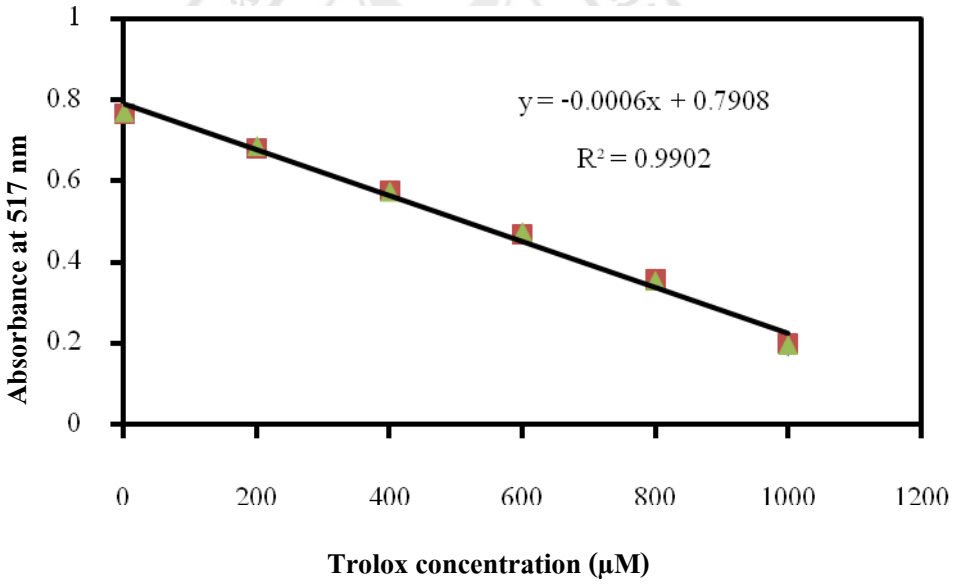


Figure C.2.1 Standard curve of antioxidant activity as trolox (µM)

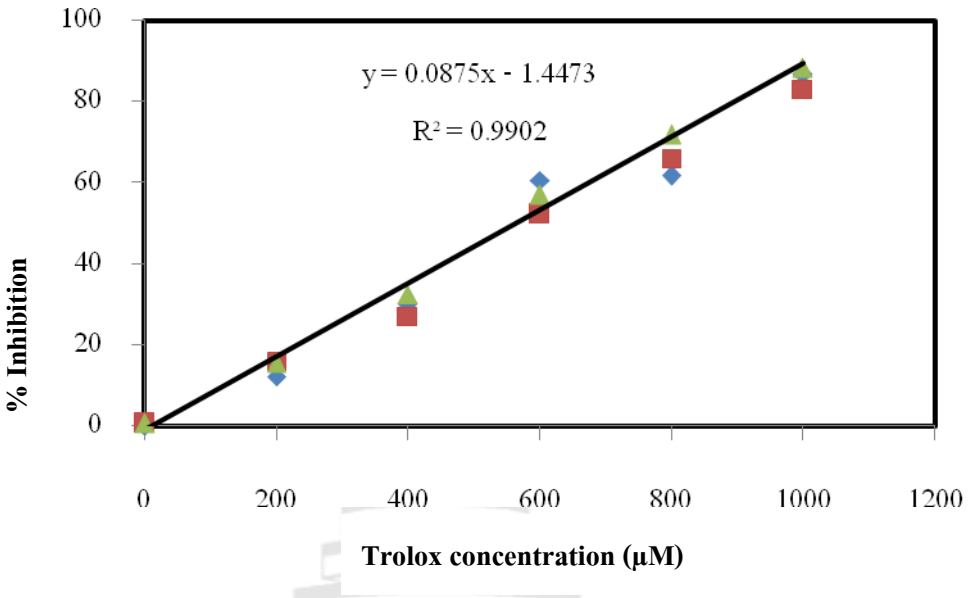


Figure C.2.1 Standard curve of antioxidant activity as % inhibition

C.3 Standard curve of catechins

HPLC method was conducted according to ISO:14502-2 (2005). Various concentrations (0.2, 1, 5, 20, 40, 60, 80 and 100 ppm) of standard working solution (C, CG, EC, EGC, ECG, GC, GCG, EGCG, G and CF) were prepared from the standard stock solution (500 ppm). Then, 1 ml aliquot of a solution was filtered through a millipore filter (0.45 µm) and the filtrate was collected in a small amber vial. Subsequently, 10 µl of the filtrate was injected to the HPLC. The concenctration was then plotted versus the obtained peak area as shown in Figure C.3.1-C.3.10.

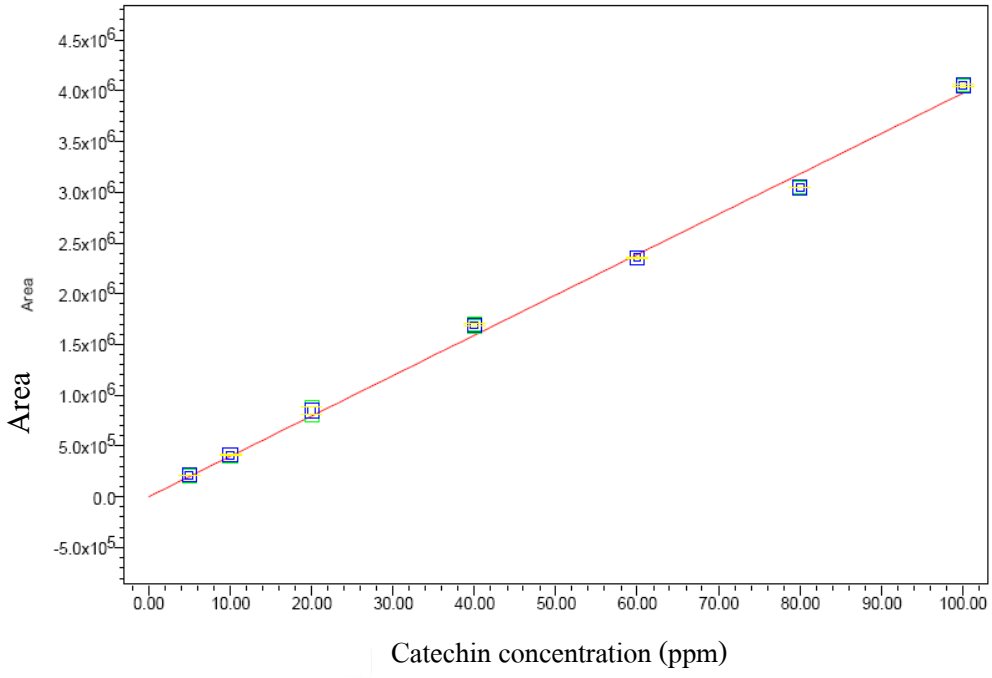


Figure C.3.1 Standard curve of catechin (C) (ppm)

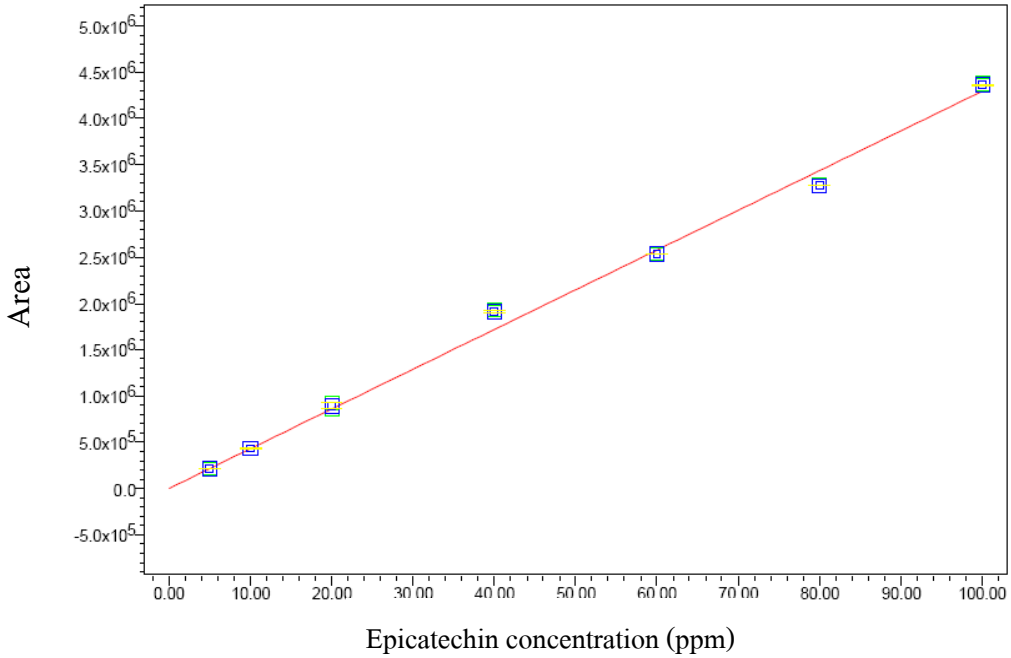


Figure C.3.2 Standard curve of epicatechin (EC) (ppm)

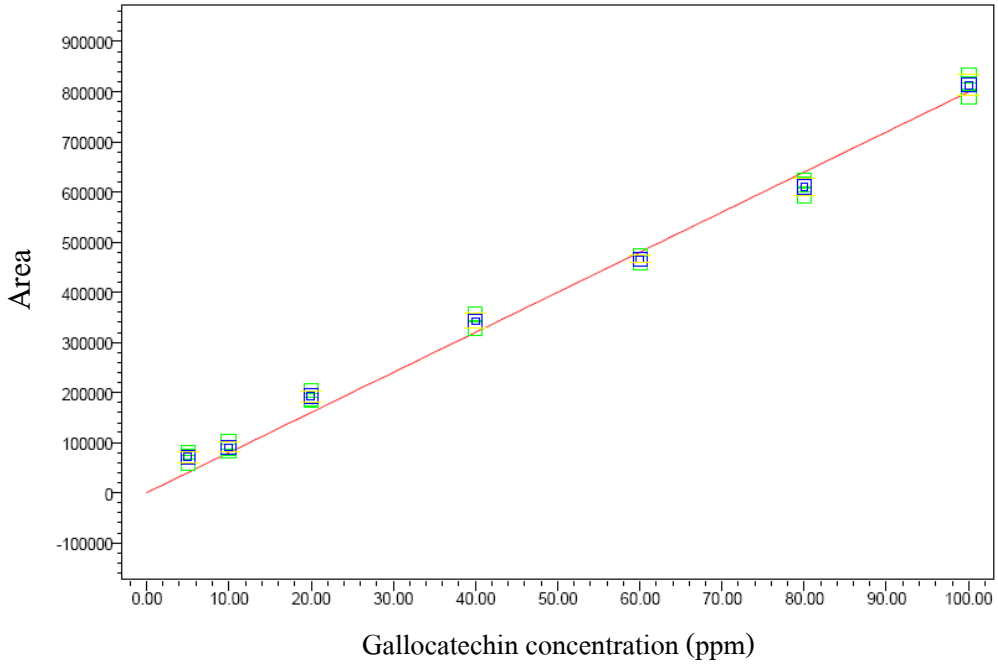


Figure C.3.3 Standard curve of gallocatechin (GC) (ppm)

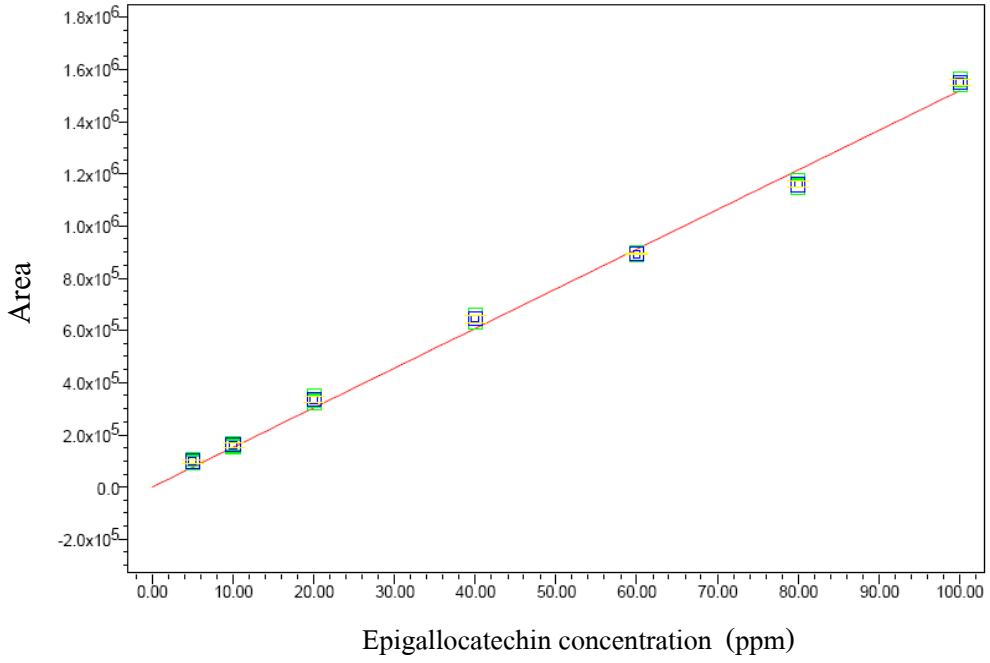


Figure C.3.4 Standard curve of epigallocatechin (EGC)

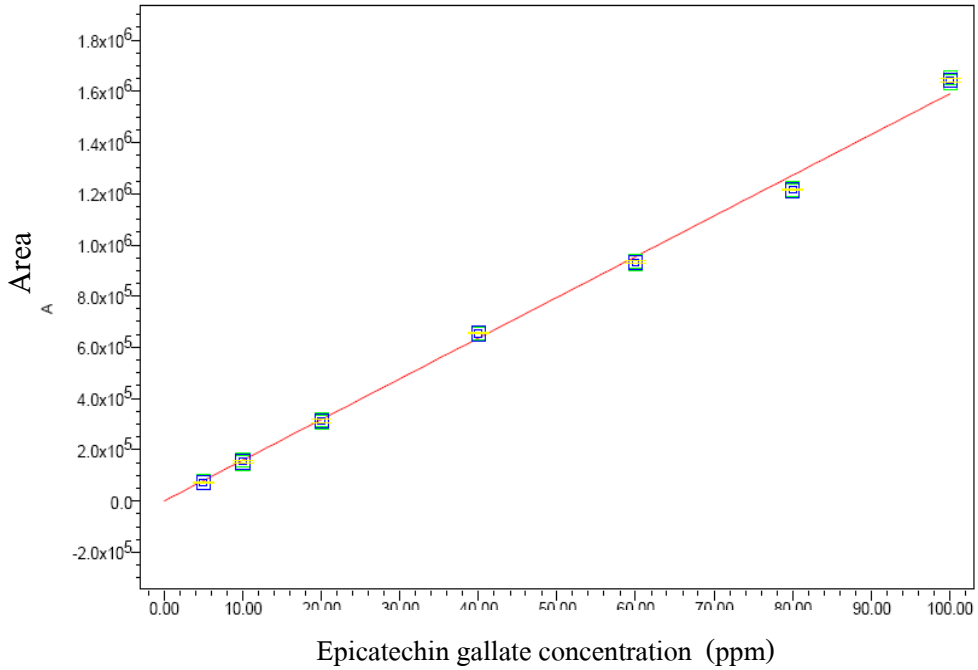


Figure C.3.5 Standard curve of epicatechin gallate (ECG) (ppm)

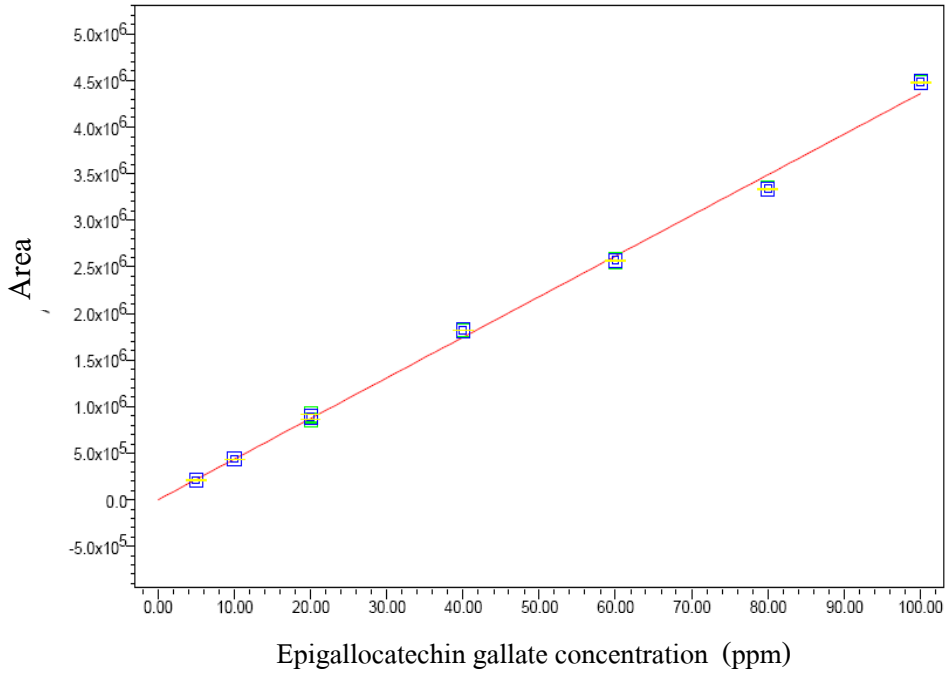


Figure C.3.6 Standard curve of epigallocatechin gallate (EGCG) (ppm)

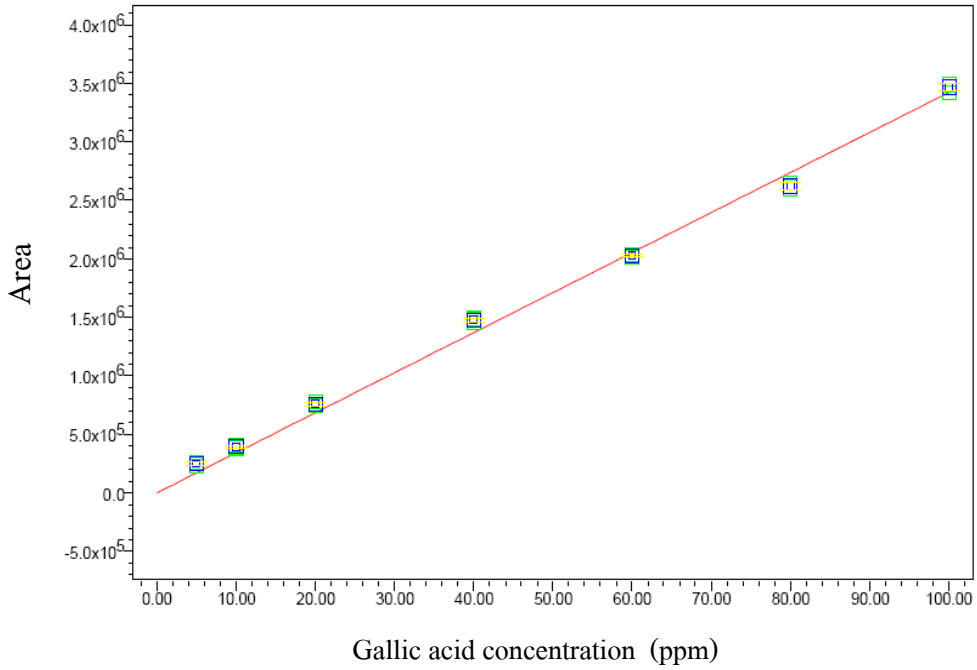


Figure C.3.7 Standard curve of gallic acid (G) (ppm)

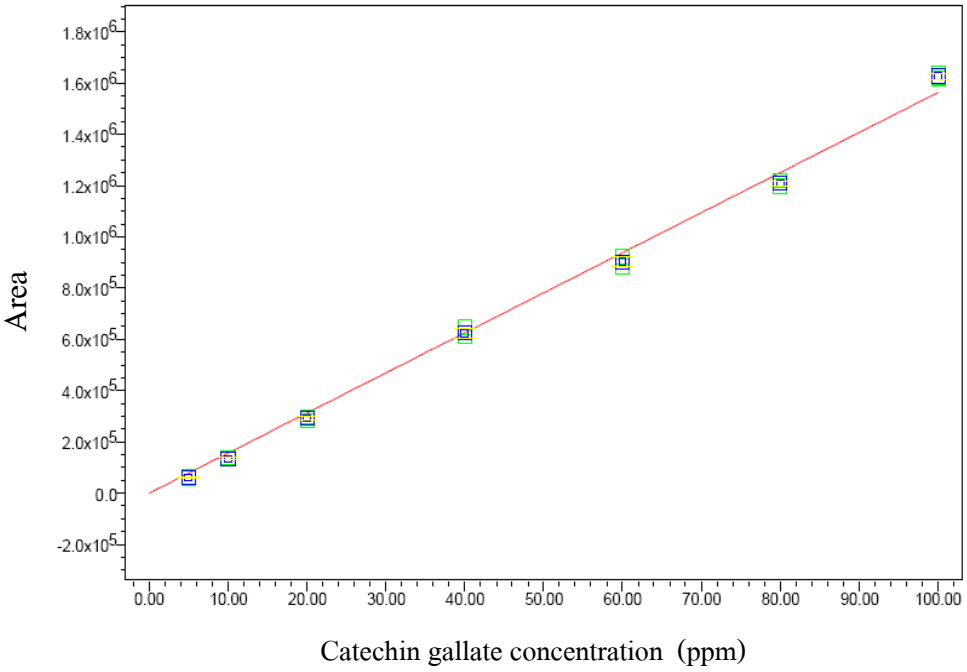


Figure C.3.8 Standard curve of catechin gallate (CG) (ppm)

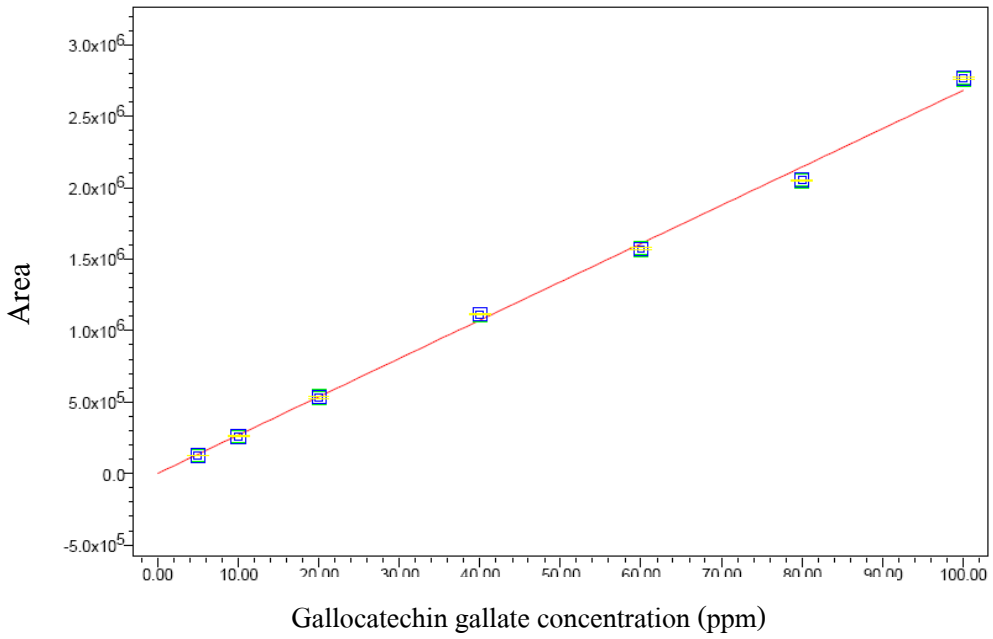


Figure C.3.9 Standard curve of gallocatechin gallate (GCG) (ppm)

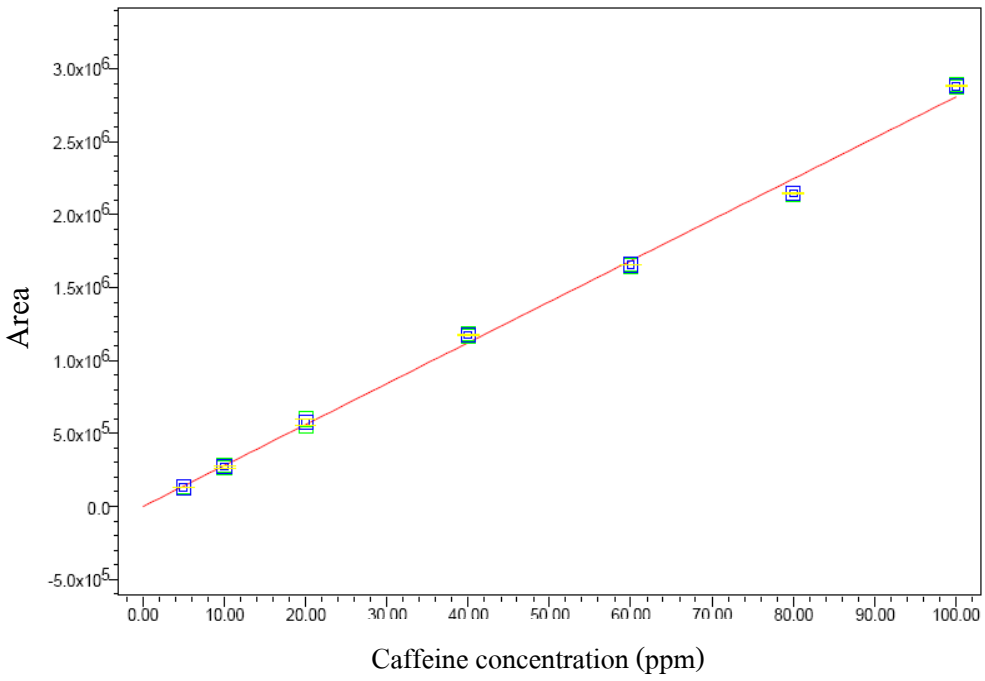


Figure C.3.10 Standard curve of caffeine (CF) (ppm)

C.4 Standard curve of peroxide value

Peroxide value was determined according to the method of Honero & Mendez (2001). Briefly, 0.34 g of Fe (III) was dissolved with 2 ml of 1% HCl to get the stock standard Fe (III) of 1040 µg/ml. Then, a working solution was prepared freshly by mixing 0.15 ml of the stock Fe (III) standard solution with chloroform : acetic acid (3:2) mixture to obtain the concentration of 10.4 µg/ml of working solution. The standard curve was plotted each day of analysis by diluting the working stock solution with different volumes of chloroform-acetic acid (3:2) in order to get the concentration of 2.5, 5, 7.5, and 10 µg/ml. Each concentration (0.05 ml) was mixed with 50 µl of 30% ammonium thiocyanate solution and vortexed for 2 to 4 s. Then, 50 µl of 1 % ferrous iron solution was added and vortexed for 2 to 4 s. The optical density of the obtained solution was determined by spectrophotometer (Lamda 35, Perkin Elmer Life and Analytical Sciences, Inc, USA) at the wavelength of 470 nm and the standard curve was plotted as shown in Figure C.4.

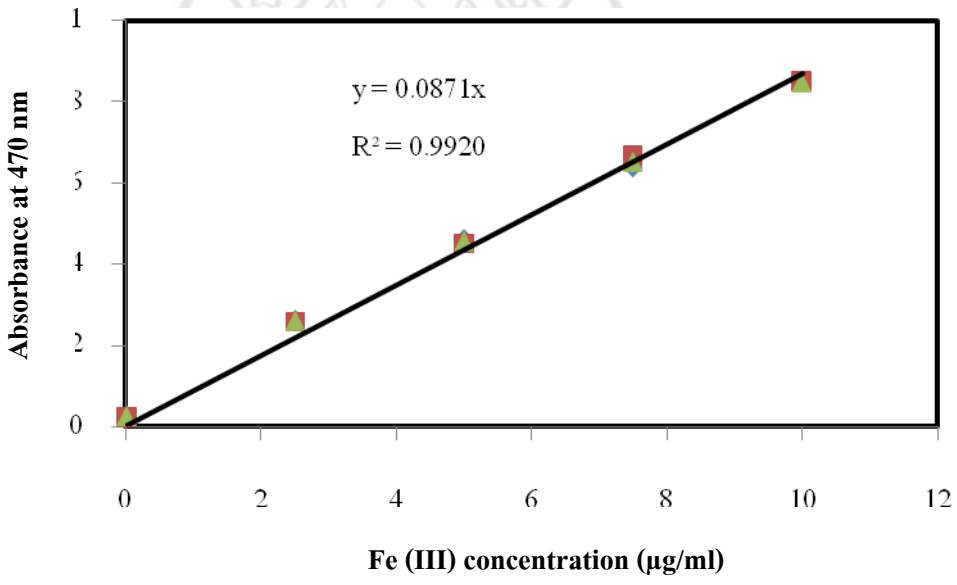


Figure C.4 Standard curve of peroxide value as Fe (III) (µg/ml)

C.5. Standard curve of TBARS

TBARS value was determined according to the method of Juntachote *et al,* (2007). Briefly, 167 µl of 1,1,3,3-Tetraethoxypropane (TEP) was diluted with 100 ml of water to get 1 mM stock TEP solution. A working solution was prepared freshly by the hydrolisis of 1 ml of TEP stock solution in 50 ml of 1 % hydrochloric acid. The resulting MAD standard solution was further dilluted with 1 % hydrochloric acid to yield concentration of 1, 2, 3, 4 and 5 nmol/ml, respectively. Each concentration (5 ml) was mixed with 5 mL of 0.02 M TBA solution and heated in a 95 °C bath for 20 minutes. Then, the absorbance was read at 532 nm by UV-Visible spectrofotometer (Lamda 35, Perkin Elmer Life and Analytical Sciences, Inc, USA). The standard curve was plotted as shown in Figure C.5.

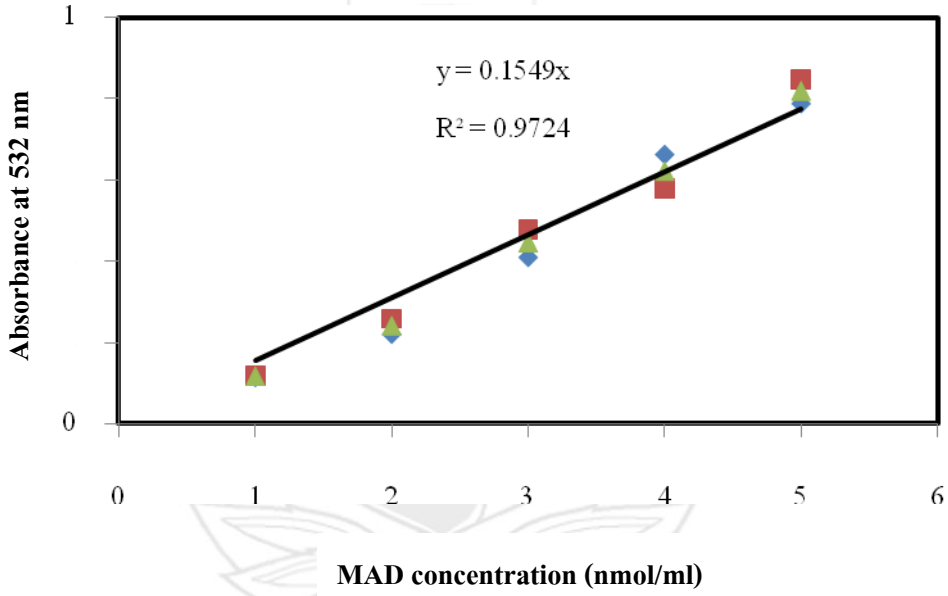


Figure C.5 Standard curve of TBARS value as malonaldehyde (nmol/ml)

APPENDIX D

HPLC ANALYSIS

D.1 HPLC of the Standard and the Sample

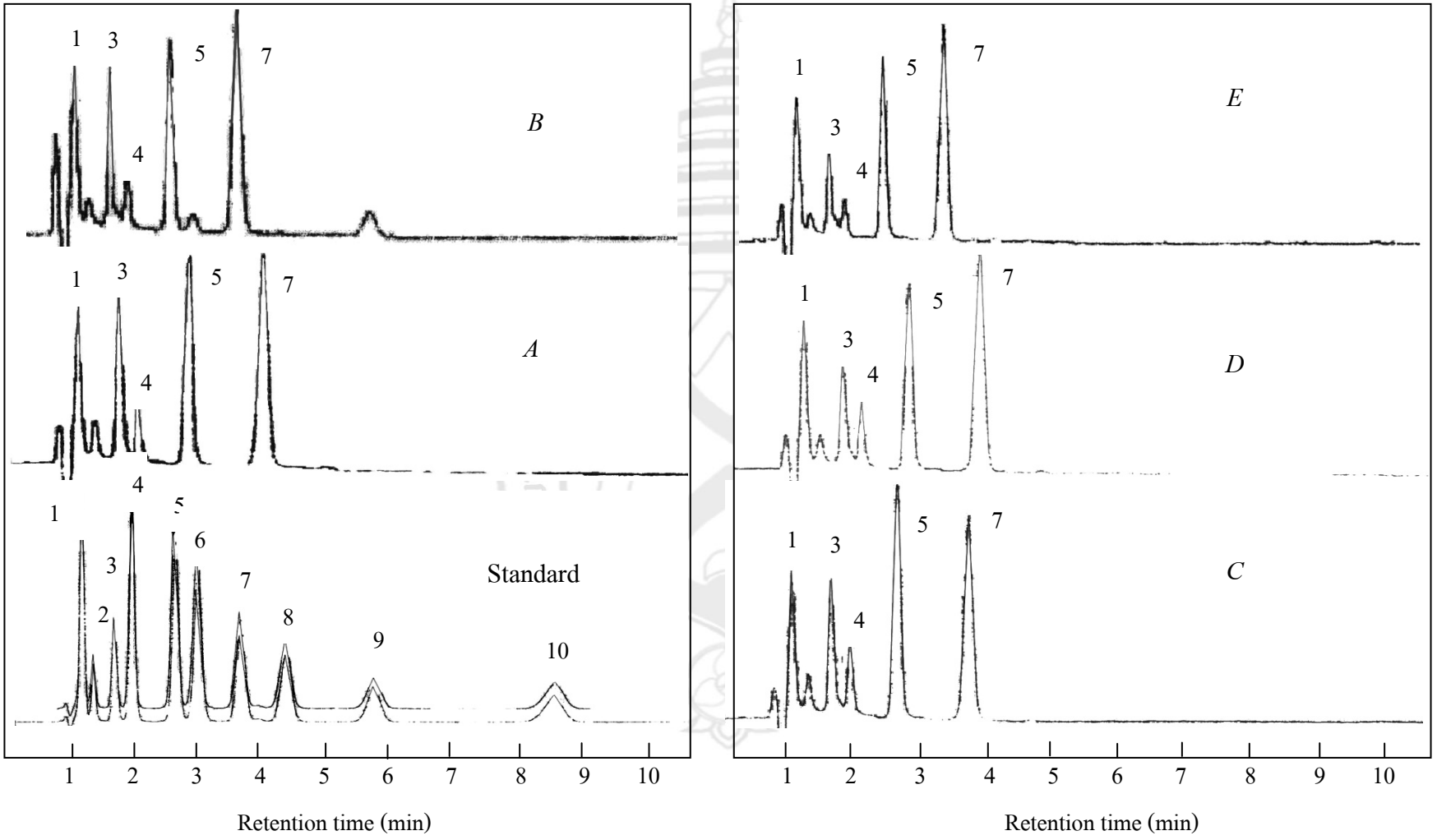


Figure D.1 The chromatogram of the standard catechins and the samples. Peaks 1 : G, 2 : GC, 3 : EGC, 4 : C, 5 : EC, 6 : EGCG, 7 : CF, 8 : GCG, 9 : ECG and 10 : CG

Table D.1 The retention time of the standard catechins and the samples

Compounds	**Retention time (min)**					
	Standard	***A***	***B***	***C***	***D***	***E***
G	1.111	1.060	1.056	1.055	1.058	1.058
GC	1.289	-	-	-	-	-
EGC	1.628	1.641	1.615	1.625	1.630	1.619
C	1.904	1.915	1.887	1.898	1.902	-
EC	2.591	2.615	2.558	2.585	2.595	2.561
EGCG	2.944	-	-	-	-	-
CF	3.664	3.668	3.601	3.640	3.651	3.604
GCG	4.363	-	-	-	-	-
ECG	5.787	-	-	-	-	-
CG	8.672	-	-	-	-	-

'

APPENDIX E

STATISTIC ANALYSIS

Table E.1 TPC and DPPH scavenging activity of commercial assam green tea infusion

Samples	TPC	DPPH Scavenging Activity
A	23.50[a]	90.10[a]
B	20.18[b]	82.81b
C	17.39[c]	77.60[c]
D	15.95[d]	75.39[d]
E	15.86[d]	73.96[d]

[a-d] Means with different superscript letters within a column are significantly different at P ≤ 0.05.

Table E.2 Antimicrobial activity of commercial assam green tea infusion against *S. aureus*

Sample	Concentration (mg/ml)								
	25	50	75	100	125	150	175	200	250
	inhibition zones (mm)								
A	6.00[aw]	7.50[bw]	8.30[cx]	9.20[dx]	10.53[dy]	11.80[cy]	12.50[cy]	14.27[ez]	16.33[ez]
B	6.00[aw]	6.40[aw]	7.90[bx]	8.50[cx]	10.27[cy]	11.60[cy]	12.30[cy]	13.33[dz]	14.33[dz]
C	6.00[aw]	6.30[aw]	7.30[bx]	8.07[cx]	9.97[cy]	11.10[cy]	11.70[by]	12.00[cz]	13.17[cz]
D	6.00[aw]	6.30[aw]	6.80[ax]	7.20[bx]	7.70[by]	9.10[by]	11.23[by]	11.93[bz]	12.32[bz]
E	6.00[aw]	6.10[aw]	6.30[ax]	6.40[ax]	6.60[ay]	6.83[ay]	7.73[ay]	8.57[az]	10.50[az]

[a-d] Means with different superscript letters within a column are significantly different at P ≤ 0.05.

[w-z] Means with different superscript letters within a row are significantly different at P ≤ 0.05.

Table E.3 Antimicrobial activity of commercial assam green tea infusion against *L. monocytogenes*

Sample	Concentration (mg/ml)								
	25	50	75	100	125	150	175	200	250
	Inhibition zones (mm)								
A	6.00^{aw}	6.00^{aw}	7.30^{bx}	8.50^{cx}	9.23^{cy}	10.77^{dy}	11.40^{dy}	12.90^{dz}	14.00^{ez}
B	6.00^{aw}	6.00^{aw}	6.30^{ax}	7.50^{bx}	8.90^{by}	10.30^{dy}	10.73^{cy}	12.07^{dz}	12.67^{dz}
C	6.00^{aw}	6.00^{aw}	6.20^{ax}	7.30^{bx}	8.23^{cy}	9.67^{cy}	10.23^{cy}	11.17^{cz}	11.83^{cz}
D	6.00^{aw}	6.00^{aw}	6.15^{ax}	6.70^{ax}	7.47^{ay}	8.23^{by}	9.30^{by}	9.83^{bz}	10.30^{bz}
E	6.00^{aw}	6.00^{aw}	6.13^{ax}	6.50^{ax}	7.00^{ay}	7.77^{ay}	8.83^{ay}	8.83^{az}	9.10^{az}

$^{a-d}$ Means with different superscript letters within a column are significantly different at $P \leq 0.05$.

$^{w-z}$ Means with different superscript letters within a row are significantly different at $P \leq 0.05$.

Table E.4 Antimicrobial activity of commercial assam green tea infusion against *S. typhimurium*

Sample	Concentration (mg/ml)								
	25	50	75	100	125	150	175	200	250
	Inhibition zones (mm)								
A	6.00^{aw}	6.00^{aw}	6.00^{ax}	6.50^{bx}	7.30^{by}	8.20^{by}	9.80^{cy}	10.53^{cz}	11.00^{dz}
B	6.00^{aw}	6.00^{aw}	6.00^{ax}	6.00^{ax}	6.00^{ay}	6.00^{ay}	7.33^{by}	8.53^{bz}	10.00^{cz}
C	6.00^{aw}	6.00^{aw}	6.00^{ax}	6.00^{ax}	6.00^{ay}	6.00^{ay}	7.10^{by}	8.23^{bz}	8.83^{bz}
D	6.00^{aw}	6.00^{aw}	6.00^{ax}	6.00^{ax}	6.00^{ay}	6.00^{ay}	6.00^{ay}	6.00^{az}	7.50^{az}
E	6.00^{aw}	6.00^{aw}	6.00^{ax}	6.00^{ax}	6.00^{ay}	6.00^{ay}	6.00^{ay}	6.00^{az}	7.17^{az}

$^{a-d}$ Means with different superscript letters within a column are significantly different at $P \leq 0.05$.

$^{w-z}$ Means with different superscript letters within a row are significantly different at $P \leq 0.05$.

Table E.5 Antimicrobial activity of commercial assam green tea infusion against *E. coli*

Sample	Concentration (mg/ml)								
	25	50	75	100	125	150	175	200	250
	Inhibition zones (mm)								
A	6.00^{aw}	6.00^{aw}	6.00^{ax}	6.00^{ax}	6.00^{ay}	6.00^{ay}	6.00^{ay}	6.00^{az}	8.00^{cz}
B	6.00^{aw}	6.00^{aw}	6.00^{ax}	6.00^{ax}	6.00^{ay}	6.00^{ay}	6.00^{ay}	6.00^{az}	7.33^{bz}
C	6.00^{aw}	6.00^{aw}	6.00^{ax}	6.00^{ax}	6.00^{ay}	6.00^{ay}	6.00^{ay}	6.00^{az}	6.83^{az}
D	6.00^{aw}	6.00^{aw}	6.00^{ax}	6.00^{ax}	6.00^{ay}	6.00^{ay}	6.00^{ay}	6.00^{az}	6.00^{az}
E	6.00^{aw}	6.00^{aw}	6.00^{ax}	6.00^{ax}	6.00^{ay}	6.00^{ay}	6.00^{ay}	6.00^{az}	6.00^{az}

$^{a-d}$ Means with different superscript letters within a column are significantly different at $P \leq 0.05$.

$^{w-z}$ Means with different superscript letters within a row are significantly different at $P \leq 0.05$.

Table E.6 *S. aureus* population in different concentrations of assam green tea infusion after 24 hrs incubation at 35 °C

Conc.	Sample				
(mg/ml)	*A*	*B*	*C*	*D*	*E*
25	0.75^{aw}	0.85^{ax}	2.70^{ax}	3.20^{ay}	3.60^{az}
50	0.00^{bw}	0.00^{bx}	1.40^{bx}	2.25^{by}	2.50^{bz}
75	0.00^{bw}	0.00^{bx}	0.00^{cx}	0.00^{cy}	0.00^{cz}
100	0.00^{bw}	0.00^{bx}	0.00^{cx}	0.00^{cy}	0.00^{cz}
125	0.00^{bw}	0.00^{bx}	0.00^{cx}	0.00^{cy}	0.00^{cz}
150	0.00^{bw}	0.00^{bx}	0.00^{cx}	0.00^{cy}	0.00^{cz}
175	0.00^{bw}	0.00^{bx}	0.00^{cx}	0.00^{cy}	0.00^{cz}
200	0.00^{bw}	0.00^{bx}	0.00^{cx}	0.00^{cy}	0.00^{cz}
225	0.00^{bw}	0.00^{bx}	0.00^{cx}	0.00^{cy}	0.00^{cz}
250	0.00^{bw}	0.00^{bx}	0.00^{cx}	0.00^{cy}	0.00^{cz}

$^{a-d}$ Means with different superscript letters within a column are significantly different at $P \leq 0.05$.

$^{w-z}$ Means with different superscript letters within a row are significantly different at $P \leq 0.05$.

Table E.7 *L. monocytogenes* population in different concentrations of assam green tea infusion after 24 hrs incubation at 35 °C

Conc.	Population (Log CFU/ml)				
(mg/ml)	*A*	*B*	*C*	*D*	*E*
25	4.60^{aw}	5.40^{ax}	6.20^{ax}	6.40^{ay}	6.60^{az}
50	3.10^{bw}	3.70bx	5.20^{bx}	5.50^{by}	5.80^{bz}
75	0.00^{cw}	0.00^{cx}	2.35^{cx}	2.6^{cy}	4.30^{cz}
100	0.00^{cw}	0.00^{cx}	0.00^{dx}	0.00^{dy}	3.80^{dz}
125	0.00^{cw}	0.00^{cx}	0.00^{dx}	0.00^{dy}	2.90^{ez}
150	0.00^{cw}	0.00^{cx}	0.00^{dx}	0.00^{dy}	0.00^{fz}
175	0.00^{cw}	0.00^{cx}	0.00^{dx}	0.00^{dy}	0.00^{fz}
200	0.00^{cw}	0.00^{cx}	0.00^{dx}	0.00^{dy}	0.00^{fz}
225	0.00^{cw}	0.00^{cx}	0.00^{dx}	0.00^{dy}	0.00^{fz}
250	0.00^{cw}	0.00^{cx}	0.00^{dx}	0.00^{dy}	0.00^{fz}

$^{a-e}$ Means with different superscript letters within a column are significantly different at $P \leq 0.05$.

$^{w-z}$ Means with different superscript letters within a row are significantly different at $P \leq 0.05$.

Table E.8 *S. typhimurium* population in different concentrations of assam green tea infusion after 24 hrs incubation at 35 °C

Conc.	Population (Log CFU/ml)				
(mg/ml)	*A*	*B*	*C*	*D*	*E*
25	7.70^{aw}	8.00^{ax}	7.80^{ax}	7.90^{ay}	8.00^{az}
50	4.70^{bw}	5.10^{bx}	5.60^{bx}	6.10^{by}	6.50^{bz}
75	3.50^{bw}	4.30^{bx}	4.70^{bx}	5.20^{by}	5.40^{bz}
100	3.00^{cw}	3.50^{cx}	3.70^{cx}	4.10^{cy}	4.30^{cz}
125	2.10^{cw}	2.42^{cx}	2.70^{cx}	3.20^{cy}	3.50^{cz}
150	0.00^{dw}	0.00^{dx}	2.20^{dx}	2.30^{dy}	2.50^{dz}
175	0.00^{dw}	0.00^{dx}	0.00^{ex}	0.00^{ey}	0.00^{ez}
200	0.00^{dw}	0.00^{dx}	0.00^{ex}	0.00^{ey}	0.00^{ez}
225	0.00^{dw}	0.00^{dx}	0.00^{ex}	0.00^{ey}	0.00^{ez}
250	0.00^{dw}	0.00^{dx}	0.00^{ex}	0.00^{ey}	0.00^{ez}

$^{a-e}$ Means with different superscript letters within a column are significantly different at $P \leq 0.05$.

$^{w-z}$ Means with different superscript letters within a row are significantly different at $P \leq 0.05$.

Table E.9 *E. coli* population in different concentration of assam green tea infusion after 24 hrs incubation at 35 °C

Conc.	Population (Log CFU/ml)				
(mg/ml)	*A*	*B*	*C*	*D*	*E*
25	7.50^{aw}	8.00^{ax}	7.80^{ax}	7.90^{ay}	8.00^{az}
50	6.20^{aw}	6.70^{ax}	6.70^{ax}	7.10^{ay}	7.40^{az}
75	5.50^{bw}	5.80^{bx}	5.90^{bx}	6.80^{by}	6.80^{bz}
100	4.40^{bw}	4.80^{bx}	4.80^{bx}	6.10^{by}	6.40^{bz}
125	3.20^{cw}	3.40^{cx}	3.70^{cx}	5.60^{cy}	5.70^{cz}
150	2.50^{cw}	2.70^{cx}	2.90^{cx}	4.30^{cy}	4.60^{cz}
175	1.90^{cw}	2.10^{cx}	2.30^{cx}	3.20^{cy}	3.70^{cz}
200	1.50^{dw}	1.75^{dx}	$1.81d^{x}$	2.90^{dy}	3.40^{dz}
225	0.00^{ew}	0.00^{ex}	0.00^{ex}	1.25^{dy}	2.13^{dz}
250	0.00^{ew}	0.00^{ex}	0.00^{ex}	0.00^{ey}	0.00^{ez}

$^{a-e}$ Means with different superscript letters within a column are significantly different at $P \leq 0.05$.

$^{w-z}$ Means with different superscript letters within a row are significantly different at $P \leq 0.05$.

Table E.10 Reduction of *S. aureus* in watermelon juice containing assam green tea infusion after 7 days storage at 35 °C

Samples	Reduction (Log CFU/ml)							
	Storage time (d)							
	0	1	2	3	4	5	6	7
Ctrl	8.03^{as}	8.00^{as}	7.97^{as}	7.95^{as}	7.92^{as}	7.89^{as}	7.86^{as}	7.83^{as}
A	8.03^{as}	3.45^{bt}	1.78^{bu}	0.00^{cv}	0.00^{bw}	0.00^{bx}	0.00^{by}	0.00^{bz}
B	8.03^{as}	4.50^{ct}	2.29^{cu}	0.70^{bv}	0.00^{bw}	0.00^{bx}	0.00^{by}	0.00^{bz}

$^{a-c}$ Means with different superscript letters within a column are significantly different at $P \leq 0.05$.

$^{s-z}$ Means with different superscript letters within a row are significantly different at $P \leq 0.05$.

Table E.11 Reduction of *L. monocytogenes* in watermelon juice containing assam green tea infusion after 7 days storage at 35 °C

Samples	Reduction (Log CFU/ml)							
	Storage time (d)							
	0	1	2	3	4	5	6	7
Ctrl	8.16 ^{as}	7.62 ^{as}	7.60^{as}	7.57 ^{as}	7.55 ^{as}	7.55 ^{as}	7.50 ^{as}	7.47 ^{as}
A	8.16 ^{as}	1.90 ^{bt}	0.00^{bu}	0.00 ^{bv}	0.00 ^{bw}	0.00 ^{bx}	0.00 ^{by}	0.00 ^{bz}
B	8.16 ^{as}	2.04 ^{bt}	0.70^{bu}	0.00 ^{bv}	0.00 ^{bw}	0.00 ^{bx}	0.00 ^{by}	0.00 ^{bz}

$^{a-c}$ Means with different superscript letters within a column are significantly different at $P \leq 0.05$.

$^{s-z}$ Means with different superscript letters within a row are significantly different at $P \leq 0.05$.

Table E.12 Reduction of *S. typhimurium* in watermelon juice containing assam green tea infusion after 7 days storage at 35 °C

Samples	Reduction (Log CFU/ml)							
	Storage time (d)							
	0	1	2	3	4	5	6	7
Ctrl	8.09 ^{as}	7.93 ^{as}	7.93 ^{as}	7.92 ^{as}	7.91 ^{as}	7.91 ^{as}	7.9 ^{as}	7.9 ^{as}
A	8.09 ^{as}	6.99 ^{ct}	4.33 ^{bu}	2.51 ^{bv}	0.70 ^{bw}	0.00 ^{bw}	0.00^{bw}	0.00^{bs}
B	8.09 ^{as}	7.23 ^{bt}	4.61 ^{bu}	2.94 ^{bv}	1.00 ^{bw}	0.00 ^{bx}	0.00^{bx}	0.00^{bx}

$^{a-c}$ Means with different superscript letters within a column are significantly different at $P \leq 0.05$.

$^{s-z}$ Means with different superscript letters within a row are significantly different at $P \leq 0.05$.

Table E.13 Reduction of *E. coli* in watermelon juice containing assam green tea infusion after 7 days storage at 35 °C

Samples	Reduction (Log CFU/ml)							
	Storage time (d)							
	0	1	2	3	4	5	6	7
Ctrl	7.98 ^{as}	7.97 ^{as}	7.95 ^{as}	7.95 ^{as}	7.93 ^{as}	7.93 ^{as}	7.92 ^{as}	7.92 ^{as}
A	7.98 ^{as}	6.30 ^{bs}	5.6 ^{bs}	4.29 ^{bs}	3.64 ^{bs}	1.24 ^{bs}	0.00 ^{bs}	0.00 ^{bs}
B	7.98 ^{as}	6.39 ^{bs}	5.8 ^{bs}	4.47 ^{bs}	3.88 ^{bs}	1.44 ^{bs}	0.00 ^{bs}	0.00 ^{bs}

$^{a-c}$ Means with different superscript letters within a column are significantly different at $P \leq 0.05$.

$^{s-z}$ Means with different superscript letters within a row are significantly different at $P \leq 0.05$.

Table E.14 Population of *S. aureus* in cooked beef containing assam green tea infusion after 7 days incubation at 35 °C

Storage time (d)	Population (Log CFU/g)					
	Ctrl	*A*		*B*		BHA/BHT
		125	250	125	250	
0	5.11 ^{au}	5.06 ^{au}	5.04 ^{au}	5.08 ^{au}	5.07 ^{au}	5.11 ^{au}
1	5.10 ^{au}	2.30 ^{bx}	0.00 ^{bw}	2.80 ^{bx}	0.00 ^{bw}	3.90 ^{bw}
2	5.10 ^{au}	0.00 ^{cv}	0.00 ^{bw}	0.00 ^{cv}	0.00 ^{bw}	2.30 ^{bv}
3	5.10 ^{au}	0.00 ^{cv}	0.00 ^{bw}	0.00 ^{cv}	0.00 ^{bw}	0.00 ^{dv}
4	5.10 ^{au}	0.00 ^{cv}	0.00 ^{bw}	0.00 ^{cv}	0.00 ^{bw}	0.00 ^{dv}
5	5.09 ^{au}	0.00 ^{cv}	0.00 ^{bw}	0.00 ^{cv}	0.00 ^{bw}	0.00 ^{dv}
6	5.09 ^{au}	0.00 ^{cv}	0.00 ^{bw}	0.00 ^{cv}	0.00 ^{bw}	0.00 ^{dv}
7	5.09 ^{au}	0.00 ^{cv}	0.00 ^{bw}	0.00 ^{cv}	0.00 ^{bw}	0.00 ^{dv}

$^{a-c}$ Means with different superscript letters within a column are significantly different at $P \leq 0.05$.

$^{u-x}$ Means with different superscript letters within a row are significantly different at $P \leq 0.05$.

Table E.15 Population of *L. monocytogenes* in cooked beef containing assam green tea infusion after 7 days incubation at 35 °C

Storage time (d)	Population (Log CFU/g)					
	Ctrl	*A*		*B*		BHA/BHT
		125	250	125	250	
0	15.00 ^{au}	14.50 ^{au}	13.00 ^{au}	14.5 ^{au}	14.5 ^{au}	14.5 ^{au}
1	14.88 ^{au}	3.60 ^{bw}	3.25 ^{bw}	3.72 ^{bw}	3.40 ^{bw}	4.12 ^{bv}
2	14.65 ^{au}	0.48 ^{cv}	0.08 ^{cv}	0.57 ^{cv}	0.27 ^{cv}	0.82 ^{cv}
3	14.61 ^{au}	0.03 ^{dv}	0.00 ^{cv}	0.10 ^{cv}	0.00 ^{dv}	0.15 ^{cv}
4	14.30 ^{au}	0.00 ^{dv}	0.00 ^{cv}	0.00 ^{dv}	0.00 ^{dv}	0.00 ^{dv}
5	14.20 ^{au}	0.00 ^{dv}	0.00 ^{cv}	0.00 ^{dv}	0.00 ^{dv}	0.00 ^{dv}
6	14.20 ^{au}	0.00 ^{dv}	0.00 ^{cv}	0.00 ^{dv}	0.00 ^{dv}	0.00 ^{dv}
7	14.15 ^{au}	0.00 ^{dv}	0.00 ^{cv}	0.00 ^{dv}	0.00 ^{dv}	0.00 ^{dv}

$^{a-d}$ Means with different superscript letters within a column are significantly different at $P \leq 0.05$.

$^{u-w}$ Means with different superscript letters within a row are significantly different at $P \leq 0.05$.

Table E.16 Population of *S. typhimurium* in cooked beef containing assam green tea infusion after 7 days incubation at 35 °C

Storage time (d)	Population (Log CFU/g)					
	Ctrl	*A*		*B*		BHA/BHT
		125	250	125	250	
0	5.31 ^{au}	5.29 ^{au}	5.24 ^{au}	5.29 ^{au}	5.25 ^{au}	5.31 ^{au}
1	5.28 ^{au}	4.76 ^{av}	0.00 ^{bw}	4.83 ^{bv}	0.00 ^{bw}	4.87 ^{bv}
2	5.28 ^{au}	2.36 ^{bw}	0.00 ^{bw}	2.89 ^{cw}	0.00 ^{bw}	4.09 ^{bv}
3	5.26 ^{au}	0.00 ^{cw}	0.00 ^{bw}	0.00 ^{dw}	0.00 ^{bw}	3.00 ^{cv}
4	5.26 ^{au}	0.00 ^{cw}	0.00 ^{bw}	0.00 ^{dw}	0.00 ^{bw}	0.00 ^{dw}
5	5.26 ^{au}	0.00 ^{cw}	0.00 ^{bw}	0.00 ^{dw}	0.00 ^{bw}	0.00 ^{dw}
6	5.26 ^{au}	0.00 ^{cw}	0.00 ^{bw}	0.00 ^{dw}	0.00 ^{bw}	0.00 ^{dw}
7	5.26 ^{au}	0.00 ^{cw}	0.00 ^{bw}	0.00 ^{dw}	0.00 ^{bw}	0.00 ^{dw}

$^{a-d}$ Means with different superscript letters within a column are significantly different at $P \leq 0.05$.

$^{u-w}$ Means with different superscript letters within a row are significantly different at $P \leq 0.05$.

Table E.17 Population of *E. coli* in cooked beef containing assam green tea infusion after 7 days incubation at 35 °C

Storage time (d)	Population (Log CFU/g)					
	Ctrl	*A*		*B*		BHA/BHT
		125	250	125	250	
0	15.40 ^{au}	14.20 ^{au}	13.80 ^{au}	14.70 ^{au}	14.00 ^{au}	15.20 ^{au}
1	14.90 ^{au}	4.00 ^{bx}	2.00 ^{bz}	6.20 ^{bw}	3.10 ^{by}	8.00 ^{bv}
2	14.80 ^{au}	1.24 ^{cx}	0.23 ^{cz}	2.06 ^{cw}	0.73 ^{cy}	3.40 ^{cv}
3	14.70 ^{au}	0.03 ^{dv}	0.00 ^{dv}	0.07 ^{dv}	0.00 ^{dv}	0.11 ^{dv}
4	14.60 ^{au}	0.00 ^{dv}	0.00 ^{dv}	0.00 ^{dv}	0.00 ^{dv}	0.02 ^{dv}
5	14.60 ^{au}	0.00 ^{dv}	0.00 ^{dv}	0.00 ^{dv}	0.00 ^{dv}	0.00 ^{dv}
6	14.60 ^{au}	0.00 ^{dv}	0.00 ^{dv}	0.00 ^{dv}	0.00 ^{dv}	0.00 ^{dv}
7	14.60 ^{au}	0.00 ^{dv}	0.00 ^{dv}	0.00 ^{dv}	0.00 ^{dv}	0.00 ^{dv}

$^{a-d}$ Means with different superscript letters within a column are significantly different at $P \leq 0.05$.

$^{u-z}$ Means with different superscript letters within a row are significantly different at $P \leq 0.05$.

Table E.18 Peroxide value (meq peroxide/kg) in the cooked beef containing different concentration of assam green tea infusions and 0.02% BHA/BHT during 7 days storage at 4 °C

Storage time (d)	PV (meq peroxide/kg)									
	Ctrl	*A*				*B*				BHA/BHT
		31.3	62.5	125	250	31.3	62.5	125	250	
0	1.54 ^{au}	0.76 ^{av}	0.68 ^{av}	0.51 ^{av}	0.43 ^{av}	0.89 ^{av}	0.74 ^{av}	0.66 ^{av}	0.54 ^{av}	1.05 ^{au}
1	3.54 ^{bu}	1.81 ^{bw}	1.32 ^{bw}	0.87 ^{bw}	0.71 ^{bw}	1.91 ^{bw}	1.30 ^{bw}	0.89 ^{bw}	0.45 ^{bw}	2.76 ^{bv}
2	5.53 ^{cu}	2.96 ^{cw}	2.45 ^{cw}	1.75 ^{cx}	1.03 ^{cx}	3.12 ^{w}	2.60 ^{cw}	1.83 ^{cx}	1.15 ^{cx}	4.33 ^{cv}
3	7.35 ^{du}	3.97 ^{dw}	3.01 ^{dw}	2.71 ^{dx}	2.04 ^{dx}	4.09 ^{dw}	3.48 ^{dw}	2.83 ^{dx}	2.10 ^{dx}	5.31 ^{dv}
4	9.26 ^{eu}	4.25 ^{ew}	3.74 ^{ew}	2.95 ^{ex}	2.30 ^{e}	4.46 ^{ew}	3.76 ^{ew}	3.11 ^{ew}	2.40 ^{dx}	7.10 ^{ev}
5	11.21 ^{fu}	5.19 ^{fw}	4.25 ^{fw}	3.68 ^{fw}	2.75 ^{ew}	5.31 ^{fw}	4.38f ^{w}	3.81 ^{ew}	2.91 ^{dx}	9.30 ^{fv}
6	13.72 ^{gu}	6.12 ^{gw}	5.13 ^{gw}	4.27 ^{gw}	3.12 ^{fw}	6.32 ^{gw}	5.29 ^{gw}	4.37 ^{fw}	3.24 ^{ex}	10.12 ^{gv}
7	15.12 ^{hu}	7.22 ^{hw}	6.23 ^{hw}	5.35 ^{hw}	4.21 ^{gw}	7.41 ^{hw}	6.37 ^{hw}	5.43 ^{gw}	4.33 ^{ex}	11.13 ^{hv}

$^{a-h}$ Means with different superscript letters within a column are significantly different at $P \leq 0.05$.

$^{u-z}$ Means with different superscript letters within a row are significantly different at $P \leq 0.05$.

Table E.19 TBARS (mg malonaldehyde/kg) in the cooked beef containing different concentration of assam green tea infusions and 0.02% BHA/BHT during 7 days storage at 4 °C

Storage time (d)	TBARS (mg malonaldehyde/kg)									
	Ctrl	*A*				*B*				BHA/BHT
		31.3	62.5	125	250	31.3	62.5	125	250	
0	1.01^{au}	0.68^{au}	0.61^{au}	0.54^{au}	0.52^{au}	0.68^{au}	0.62^{au}	0.57^{au}	0.55^{au}	0.98^{au}
1	1.28^{au}	0.68^{av}	0.61^{av}	0.54^{av}	0.52^{av}	0.69^{av}	0.62^{av}	0.57^{av}	0.55^{av}	1.16^{au}
2	2.05^{bu}	0.68^{aw}	0.61^{aw}	0.54^{aw}	0.53^{aw}	0.71^{aw}	0.62^{aw}	0.57^{av}	0.55^{av}	1.31^{av}
3	3.13^{cu}	0.69^{aw}	0.65^{aw}	0.59^{aw}	0.54^{aw}	0.74^{aw}	0.63^{aw}	0.61^{aw}	0.57^{aw}	1.53^{bv}
4	4.57^{du}	0.71^{aw}	0.66^{aw}	0.6^{aw}	0.55^{aw}	0.77^{aw}	0.64^{aw}	0.62^{aw}	0.58^{aw}	1.69^{bv}
5	5.72^{eu}	0.72^{aw}	0.67^{aw}	0.62^{aw}	0.56^{aw}	0.77^{aw}	0.65^{aw}	0.63^{aw}	0.59^{aw}	1.99cv
6	6.69^{fu}	0.74^{aw}	0.68^{aw}	0.64^{aw}	0.56^{aw}	0.77^{aw}	0.67^{aw}	0.63^{aw}	0.59^{aw}	2.09 cv
7	7.66^{gu}	0.75^{aw}	0.69^{av}	0.66^{aw}	0.57^{aw}	0.78^{aw}	0.70^{av}	0.64^{aw}	0.60^{aw}	2.27 cv

$^{a-g}$ Means with different superscript letters within a column are significantly different at $P \leq 0.05$.

$^{u-w}$ Means with different superscript letters within a row are significantly different at $P \leq 0.05$.

Table E.20 ΔL^* value in the cooked beef containing different concentration of assam green tea infusions and 0.02% BHA/BHT during 7 days storage at 4 °C

Storage time (d)	ΔL^* value									
	Ctrl	*A*				*B*				BHA/BHT
		31.3	62.5	125	250	31.3	62.5	125	250	
0	0.00 ^{au}	0.00 ^{au}	0.00 ^{au}	0.00 ^{au}	0.00 ^{au}	0.00 ^{au}	0.00 ^{au}	0.00 ^{au}	0.00 ^{au}	0.00 ^{au}
1	0.03 ^{au}	0.74^{au}	0.42^{au}	0.43^{bu}	0.51^{bu}	0.46^{bu}	0.52^{bu}	0.52^{bu}	0.34^{bu}	0.08 ^{bu}
2	0.10 ^{au}	1.21 ^{av}	1.07^{bv}	0.73^{bu}	1.04^{bu}	0.86^{bu}	0.91^{bu}	0.90^{bu}	0.78^{bu}	0.22 ^{bu}
3	0.12 ^{au}	1.67 ^{bv}	1.53^{bv}	1.13^{bv}	1.50^{bv}	1.26^{cv}	1.30^{cv}	1.25^{cv}	1.21^{cv}	0.44 ^{bu}
4	0.15 ^{au}	1.94 ^{bv}	1.92^{bv}	1.48^{b}	2.02^{cv}	1.52^{cv}	1.57^{cv}	1.57^{cv}	1.58^{cv}	0.63 ^{bu}
5	0.24 ^{au}	2.12 ^{cv}	2.13^{cv}	1.93^{bv}	2.36^{cv}	1.82^{cv}	1.88^{cv}	2.00^{cv}	2.03^{dv}	0.84 ^{bu}
6	0.34 ^{au}	2.22^{cw}	2.41^{cw}	2.31^{cw}	2.61^{cw}	2.07^{dw}	2.13^{cw}	2.40^{cw}	2.36^{dw}	1.02 ^{cv}
7	0.38 ^{au}	2.38^{cw}	2.60^{cw}	2.72^{cw}	2.80^{cw}	2.22^{dw}	2.41^{cw}	2.64^{cw}	2.77^{dw}	1.25 ^{cv}

[a-d] Means with different superscript letters within a column are significantly different at $P \leq 0.05$.

[u-w] Means with different superscript letters within a row are significantly different at $P \leq 0.05$.

Table E.21 Δ *a** value in the cooked beef containing different concentration of assam green tea infusions and 0.02% BHA/BHT during 7 days storage at 4 °C

Storage time (d)	Δ *a** value									
	Ctrl	*A*				*B*				BHA/BHT
		31.3	62.5	125	250	31.3	62.5	125	250	
0	0.00 ^{au}	0.00 ^{au}	0.00 ^{au}	0.00 ^{au}	0.00 ^{au}	0.00 ^{au}	0.00 ^{au}	0.00 ^{au}	0.00 ^{au}	0.00 ^{au}
1	-0.56^{au}	-0.20^{au}	-0.25 ^{au}	-0.15^{au}	-0.03^{au}	-0.45 ^{au}	-0.36 ^{au}	-0.18 ^{au}	-0.17 ^{au}	-0.48 ^{cu}
2	-1.11 ^{bu}	-0.66^{au}	-0.47 ^{au}	-0.23 ^{au}	-0.18 ^{au}	-0.91 ^{au}	-0.55 ^{au}	-0.25a^{u}	-0.27 ^{au}	-0.94 ^{cu}
3	-1.59 ^{bu}	-0.91 ^{au}	-0.61 ^{au}	-0.32 ^{au}	-0.28 ^{au}	-1.21 ^{bu}	-0.68 ^{au}	-0.37 ^{au}	-0.36 ^{au}	-1.23 ^{cu}
4	-1.89 ^{bu}	-1.12 ^{bu}	-0.73 ^{au}	-0.42 ^{au}	-0.36 ^{au}	-1.44 ^{bu}	-0.81 ^{au}	-0.42 ^{au}	-0.43 ^{au}	-1.43 ^{cu}
5	-2.23 ^{cu}	-1.32 ^{bu}	-0.92 ^{au}	-0.52 ^{au}	-0.45 ^{au}	-1.67 ^{bu}	-1.00 ^{bu}	-0.55 ^{au}	-0.52 ^{au}	-1.61 ^{cu}
6	-2.60 ^{cu}	-1.51 ^{bu}	-1.01 ^{bu}	-0.65 ^{au}	-0.56 ^{au}	-1.84 ^{bu}	-1.14 ^{bu}	-0.71 ^{au}	-0.59 ^{au}	-1.85 ^{cu}
7	-2.89 ^{cu}	-1.62 ^{bu}	-1.13 ^{bu}	-0.77 ^{au}	-0.64 ^{au}	-1.95 ^{bu}	-1.24 ^{bu}	-0.82 ^{au}	-0.67 ^{au}	-1.99 ^{cu}

$^{a-d}$ Means with different superscript letters within a column are significantly different at $P \leq 0.05$.

$^{u-z}$ Means with different superscript letters within a row are significantly different at $P \leq 0.05$.

Table E.22 Δ *b** value in the cooked beef containing different concentration of assam green tea infusions and 0.02% BHA/BHT during 7 days storage at 4 °C

Storage time (d)	Δ *b** value									
	Ctrl	*A*				*B*				BHA/BHT
		31.3	62.5	125	250	31.3	62.5	125	250	
0	0.00 ^{au}	0.00 ^{au}	0.00 ^{au}	0.00 ^{au}	0.00 ^{au}	0.00 ^{au}	0.00 ^{au}	0.00 ^{au}	0.00 ^{au}	0.00 ^{au}
1	-0.22 ^{au}	-0.16 ^{au}	-0.14 ^{au}	-0.17 ^{au}	-0.14 ^{au}	-0.11 ^{au}	-0.20 ^{au}	-0.11 ^{au}	-0.13^{au}	-0.15 ^{au}
2	-0.47 ^{au}	-0.23 ^{au}	-0.24 ^{au}	-0.23 ^{au}	-0.20 ^{au}	-0.20 ^{au}	-0.30 ^{au}	-0.20 ^{au}	-0.25 ^{au}	-0.37 ^{au}
3	-0.62 ^{au}	-0.37 ^{au}	-0.38 ^{u}	-0.32 ^{au}	-0.25 ^{au}	-0.33 ^{au}	-0.36 ^{au}	-0.31 ^{au}	-0.33 ^{au}	-0.45 ^{au}
4	-0.76 ^{au}	-0.49 ^{au}	-0.43 ^{au}	-0.42 ^{au}	-0.35 ^{au}	-0.48 ^{au}	-0.48 ^{au}	-0.39 ^{au}	-0.43 ^{au}	-0.55 ^{au}
5	-0.84 ^{au}	-0.64 ^{au}	-0.54 ^{au}	-0.54 ^{au}	-0.40 ^{au}	-0.59 ^{au}	-0.5 ^{au} 3	-0.56 ^{au}	-0.47 ^{au}	-0.79 ^{au}
6	-1.03 ^{au}	-0.73 ^{au}	-0.64 ^{au}	-0.61 ^{au}	-0.48 ^{au}	-0.80 ^{au}	-0.72 ^{au}	-0.66 ^{au}	-0.59 ^{au}	-0.86 ^{au}
7	-1.09 ^{au}	-0.82 ^{au}	-0.78 ^{au}	-0.68 ^{au}	-0.55 ^{au}	-0.93 ^{au}	-0.81 ^{au}	-0.78 ^{au}	-0.63 ^{au}	-0.95 ^{au}

$^{a-d}$ Means with different superscript letters within a column are significantly different at $P \leq 0.05$.

$^{u-z}$ Means with different superscript letters within a row are significantly different at $P \leq 0.05$

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Kristanti, R.A.*, Sampanvejsobha, S., Punbusayakul, N., 2008, **Susceptability Testing of Commercial Green Tea in Chiang Rai Against Pathogenic Bacteria,** Tea International Conference on Tea Production and Tea Products, Abstracts Book, OS-1, November 26-28, 2008, Chiang Rai, Thailand (with *In-Press* Proceedings).