

CHAPTER VI

DISCUSSION

6.1 Effect of Heat Processing on Total Phenolic Content and Antioxidant Activities of Eggplants

Polyphenols are the large group of phytochemicals that are gaining acceptance as being responsible for the health benefits associated with fruits and vegetables. Because of their chemical structure, plant polyphenols can scavenge free radicals and inactive other pro-oxidants, and also interact with a number of biological relevance (Nisha *et al.*, 2009). It was hypothesized that heat treatment of the present study released some phytochemicals from the insoluble portion of eggplants which increase total phenolic contents and antioxidant activities. Cooking process, namely, parboiling had an effect on the antioxidant activity (DPPH radical scavenging activity) of eggplant (นันทนา และ รุ่งทิพา, 2547). Steaming or frying increased total phenolic content and antioxidant activities of most extracts from eggplants; except that of fried Ma Khuea Lueng that had maintained total phenolic content compared with the raw one. It was reported earlier that steaming at 100°C for 5, 7.5, 10 and 15 min increased the total phenolic content and antioxidant activity of 6 cultivars of eggplants (ชนิกานัญญ์, 2552). Heating agricultural products increased total antioxidant activity but decrease or no effect on the total phenolic content. For example, thermal treatment significantly increased both the phenolic content and total antioxidant activity of sweet corn (Dewanto *et al.*, 2002b) but increased only total antioxidant activity without any significant changes in total phenolic or total flavonoid content in tomato (Dewanto *et al.*, 2002a). Roy *et al.* (2009) suggested that thermal processing increased the pool of phenolics by disrupting cell membranes and cell walls of the insoluble portion of broccoli.

Frying the steamed samples namely, Ma Khuea Pro and Ma Khuea Lueng decreased their total phenolic contents and antioxidant activities; however, the same

treatment maintained the total phenolic content and antioxidant activity (except that assayed by FRAP method) of Ma Khuea Muang Glom. Anthocyanins isolated from *Solanum melongena* L. (Ma Khuea Muang Glom) could both inhibited hydroxyl radical generation and scavenged superoxide (Kaneyuki *et al.*, 1999; Noda *et al.*, 2000). It is possible to hypothesize that anthocyanins in *Solanum melongena* L. (Ma Khuea Muang Glom) may be responsible for the increasing or maintaining the total phenolic content and antioxidant activities. The flavonoids isolated from *Solanum melongena* L. also expressed their potent antioxidant activity against chromosomal aberrations induced by doxorubicin (Sudheesh *et al.*, 1999; Sadilova *et al.*, 2006). However, Harbourne *et al.* (2008) indicated that temperature could have a deleterious effect on anthocyanins. In addition, Volden *et al.* (2008) reported that blanching, boiling and steaming resulted in losses of 59%, 41% and 29%, respectively, in anthocyanin content of red cabbage. Similar result was reported by Hager *et al.* (2008) who found that processing of berries canned in water or syrup resulted in total anthocyanin losses of 42% and 51%, respectively.

The present study suggested that anthocyanin, chlorogenic and caffeic acids of Ma Khuea Muang Glom (*Solanum melongena* L.) should be responsible for the maintenance of its total phenolic content and antioxidant activities. Chlorogenic and caffeic acids are the major phenolic compounds in eggplant (Stommel and Whitaker, 2003). *Solanum melongena* L. (Ma Khuea Muang Glom) contained 154 mg/100g of chlorogenic acid and 12.8 mg/100g caffeic acid. Grilling for 4-5 min or boiling for 10 min increased the amounts of phenolic compounds known to be antioxidants such as chlorogenic and caffeic acids (Scalzo *et al.*, 2010). Similar result was reported by Re *et al.* (2002) who found that heat processing of tomato increased chlorogenic and caffeic acids. In fact, heating has been reported to increase the chemical extractability of phytochemical compounds, because of the release of phytochemicals from chromoplasts leading to an increment of concentration (Howard *et al.*, 1999). In addition, Leong and Oey (2012) recently found that heating at 98°C for 10 min increased the anthocyanin content of cherries, peaches and plums.

6.2 Antimutagenicity of Raw and Heat Processed Eggplants

The survivals of adult flies fed on both raw and heat processed eggplants were more than fifty percents and the flies had normal size. This indicated that no sample was toxic for the tester strain. In addition, the results of this study have revealed that both raw and heat processed eggplants was not mutagenic on the somatic cells of *Drosophila* tester. These eggplants have long been consumed by Thai people without any toxic indication. Therefore, this experiment confirmed that these eggplants are safe for general consumption.

The present study aimed to elucidate whether each heat processed eggplant could modulate the mutagenicity of urethane administered to *Drosophila melanogaster*. Urethane is found in very small quantities in several fermented foods and a beverage such as stone-fruit brandies, cherries and table wines (Schlatter and Luitz, 1990). It is a well known genotoxic carcinogen that is metabolically activated by the cytochrome P-450 enzyme system. Park *et al.* (1993) demonstrated that vinyl carbamate, the metabolite of urethane and ultimately responsible for its mutagenic effects, is detoxified by the conjugation with glutathione-S-transferase (Kemper *et al.*, 1995). In this study, 20 mM urethane in the *Drosophila* medium induced about 15.11 ± 1.88 total spots per wing. If number of spots per wing reduced, it was postulated that the eggplant might act as an enzyme modifier (induce of glutathione-S-transferase (GST) or an inhibitor of cytochrome P450 system). The components of eggplants might induce the activity of glutathione-S-transferase (GST) enzyme system in the phase 2 of xenobiotic metabolism which increases the detoxification of vinyl carbamate epoxide (metabolite of urethane). Phenolic compounds were shown to exhibit important health promoting functions such as antimutagenic and anticarcinogenic activity (Yang *et al.*, 2001). Some phenolics possessed potent antioxidant activity and also had anticancer/anticarcinogenic/antimutagenic activities, such as phenolic acids (e.g. chlorogenic acid, caffeic acid, ferulic acid), flavonoids (e.g. vitexin, quercetin, wogonin, genistein, catechins, isoflavones), quinones (e.g. emodin, rhein, aloe-emodin), coumarins (e.g. 7-hydroxy-coumarin), stilbenes (e.g. resveratrol), curcuminoids (e.g. curcumin), lignans, etc. (Ho *et al.*, 1994; Gao *et al.*, 2000; Owen *et al.*, 2000; Xiao *et al.*, 2000; Yang *et al.*, 2001; Tapiero *et al.*, 2002). As

mentioned above some phenolic compounds were found to be the constituents of egg plants (Stommel and Whitaker, 2003).

The results that all eggplants in this experiment were antimutagenic make them be more attractive than just a side dish for shrimp paste dip. It was found that phytosterols from *Solanum aculeatissimum* Jacq. (in the same genus and species of Ma Khuea Pro) inhibited tumor growth and metastasis in MDA-MB-231 human breast cancer cell (Awad *et al.*, 2010a) and PC-3 human prostate cancer in SCID mice (Awad *et al.*, 2001b). Solamargine and solasonine extracts from *Solanum melongena* L. (Ma Khuea Muang Glom) were toxic to human colon cancer cell line (HT 29) and human liver cancer cell line (HepG2) (Lee *et al.*, 2004). On the hand, Akanitapichat *et al.* (2010) found that pretreatment of HepG2 with 50 and 100 µg/ml of *Solanum melongena* L. (Ma Khuea Muang Glom) extract significantly increased the viability ($p < 0.05$) of *t*-BuOOH-exposed HepG2 cells

In recent years many mutations related to carcinogenesis have been found, and this has resulted in much detailed research on mutagenesis. Consequently, from the cancer-preventing point of view, an interest has also been aroused in the presence of antimutagens in foodstuffs. A lot of them have now been identified in fruits and vegetables such as eggplants, cabbages, apples and others (Inoue *et al.*, 1981, Ishii *et al.*, 1984 and Morita *et al.*, 1978). Eggplant has been used as a home treatment medicine in Japan. Also eggplant possesses antimicrobial and antitumor activity. Samaru (1989) found that epidermis, sepals or receptacles of eggplant fruit had anticarcinogenic activity. Therefore, the result that all raw eggplants in this investigation were antimutagenic was not surprised since it was previously reported that various components of eggplant show high antimutagenic properties compared to other vegetables. The details of this activity have not yet been clarified. Shinohara (1992) reported that the polyphenols in eggplant inhibited the mutagenicity of heterocyclic amines in the Ames test. Yoshikawa *et al.* (1990) previously found that the mutagenicity of broiled fish was inhibited by crude extracts of vegetables. In addition, Yoshikawa *et al.* (1996) used *Salmonella typhimurium* TA98 of the Ames test to confirm the specific antimutagens from eggplant fruit against 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P-2). The active component in eggplants might belong to some polyphenolic compounds since Shinohara (1992) reported that antimutagenic

polyphenols in eggplant juice were heat resistant which was confirmed by the results of the present investigation that the antimutagenicity of the eggplants was rather stable or increased after heat treatments. In the experiment performed by Yoshikawa *et al.* (1996), they suspected that the antimutagenicity observed in eggplant juice was due to polyphenolic compounds such as tannin which agreed with a report by Shinohara (1992).

Heat treatment could either decrease or increase the antimutagenicity of samples; it depended on sample variety and/or type of heat processing. Steaming decreased the antimutagenicity of Ma Khuea Pro but increased that of Ma Khuea Lueng and Ma Khuea Muang Glom compared with that of their corresponding raw ones. The antimutagenicity of steamed Ma Khuea Pro decreased after being fried compared with that of the corresponding raw and steamed ones. Kijja (2002) investigated on the protective effect of raw and cooked vegetables namely, cabbage, cauliflower and Thai collared (called pule) against the mutagenicity of urethane in *Drosophila melanogaster* and revealed that boiling as well as frying reduced the antimutagenicity of the vegetables. The antimutagenicity against urethane in *Drosophila melanogaster* of raw bananas namely, kluinamwaa, kluikai and kluihom (Limpichaisopon, 2002) as well as of eggplants namely, *Lycopersicon esculentum*, *Solanum melongena*, *Solanum aculeatissimum* and *Solanum torvum* (Tanruk, 2003) reduced after being boiled. In contrast, frying both steamed Ma Khuea Lueng and steamed Ma Khuea Muang Glom did not change their antimutagenicity compared with that of the corresponding raw ones; however, the same treatment decreased the antimutagenicity of Ma Khuea Muang Glom compared with that of the steamed one.

Nisha *et al.* (2009) indicated that *Solanum melongena* L. (Ma Khuea Muang Glom) had 0.53 mg/100 g of anthocyanin which had the ability to induce phase 2 detoxifying enzymes in cultured cells (Shih *et al.*, 2005). Treatment of rat liver clone 9 cells with 50 μ M anthocyanins (Shih *et al.*, 2007) and non-cancerous breast cells with 10-20 μ g/ml anthocyanins (Singletary *et al.*, 2007) enhanced their antioxidant capacity by activating glutathione-related enzymes (glutathione reductase, glutathione peroxidase) and glutathione-S-transferase as well as the activity of NAD(P)H: quinone reductase. *Solanum melongena* L. (Ma Khuea Muang Glom) also had 154 mg/100g of chlorogenic acid and 12.8 mg/100g caffeic acid (Scalzo *et al.*, 2010). It was found that

caffeic acid isolated from *Syzygium cumini* L. (Jamun or black plum) increased antioxidant biomolecule GSH and increased phase 2 detoxification (GST) (Arun *et al.*, 2011). It is warrant that anthocyanin and caffeic acid isolated from eggplants should be evaluated for their inducible the activities of glutathione-S-transferase (GST) enzyme system has possibly played an important role in reducing the reactive form of URE which can cause DNA adduct. These results confirm the proposed hypothesis that heat treatment did not affect on the antimutagenicity of eggplants owing to such anthocyanin and caffeic acid.

6.3 Consumer Benefit on Raw or Heat Processed Eggplants

The results of this study indicated that eggplants, both raw or heat processed, had some health benefit to consumers. They had high total phenolic content and antioxidant activities. Moreover, they had antimutagenicity against a standard mutagen namely, urethane. Eggplant is suitable to be consumed either being raw or heat processed which is the natural consumption habit of Thai people. The result obtained from the study on fried eggplants indicates their opportunity to be developed as health-benefit snack containing both antioxidant and antimutagenicity. It is indicated that Ma Khuea Muang Glom was superior to other eggplants.