CHAPTER III
LITERATURE REVIEW

3.1 Eggplant

It is a member of the family *Solanaceae* and genus *Solanum*. It bears a fruit of the same name, commonly used in cooking. As a *Solanaceae*, it is closely related to the tomato and potato (Tsao and Lo, 2006; Doijode, 2001). The fruit contains ascorbic acid and phenolic compounds, both of which are powerful antioxidants (Vinson *et al*., 1998). Furthermore, understanding the health benefits of eggplant has merit when considering the cholesterol-lowering effects of a portfolio diet, which has eggplant as an important fiber source (Jenkins *et al*., 2003). Interestingly, National Diabetes Education Program of NIH (National Institute of Health), Mayo Clinic and American Diabetes Association (ADA) recommend eggplant as a part of the diet for the management of type 2 diabetes (NIH, 2011; Mayoclinic, 2011; ADA, 2011).

3.1.1 *Solanum aculeatissimum* Jacq. (Cockroach berry or Ma Khuea Pro (มะเขือเปราะ) and Yellow Berried Nightshade or Ma Khuea Lueng (มะเขือเหลือง))

The main constitutents of *Solanum aculeatissimum* Jacq. are aculeatiside, diosgenin, lanosterol, β-sitosterol, solamargine, solasodine and solasonine (นันทวัน และ อรนุช, 2542). It was found that phytosterols from *Solanum aculeatissimum* Jacq. 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). In addition, Shale *et al*. (1999) indicated that hexane, methanol and water extracts from *Solanum aculeatissimum* Jacq. expressed their medium inhibitory effect on both gram-positive and gram-negative bacteria.

Cooking process, namely, parboiling had an effect on the antioxidant activity (DPPH radical scavenging activity) of eggplant (นันทนา และ รุ่งทิวา 2547). The

parboiled *Solanum aculeatissimum* Jacq. expressed its higher antioxidant activity than the raw one. In addition, steaming at 100^{o}C for 5, 7.5, 10 and 15 min increased the antioxidant activity and total phenolic content of 6 cultivars of eggplants, namely, Chao Phraya, Pro, Yao Keaw, Yao Muang, Muang Lek and Pao with longer heating time (ชนิกาญจน์ 2552).

3.1.2 *Solanum melongena* L. (Aubergine or Ma Khuea Muang Glom (มะเขือม่วงกลม)).

It is commonly known as brinjal and aubergine in India and in Europe, respectively (Sarker *et al*., 2006). *Solanum melongena* L. is also a natural source of beta-carotene that can be converted to vitamin A; therefore, it would play an important role for vision and eye health since vitamin A is recognized as a critical factor in child health and survival (Igwe *et al*., 2003).

Eggplant extracts suppressed the development of blood vessels required for tumor growth and metastasis (Matsubara *et al*., 2005). In particular, solamargine and solasonine extracts from *Solanum melongena* L. were toxic to human colon cancer cell line (HT 29) and human liver cancer cell line (HepG2) (Lee *et al*., 2004). In addition, Akanitapichat *et al.* (2010) found that treatment with 300 μM *tert*-Butylhydroperoxide (*t*-BuOOH) alone resulted in 52.33 ± 0.50% viability while pretreatment of HepG2 with 50 and 100 μg/ml of *Solanum melongena* L. extract significantly increased the viability ($p < 0.05$) of *t*-BuOOH-exposed HepG2 cells by 14.49 ± 1.14% to 44.95 ± 2.72%. Furthermore, delphinidin-3-(*p*-coumaroylrutinoside)-5-glucoside (nasunin), an anthocyanin isolated from the skin of purple eggplant fruit, could both inhibited hydroxyl radical generation and scavenged superoxide (Kaneyuki *et al*., 1999; Noda *et al*., 2000). In addition, Azevedo *et al*. (2007) suggested that mice pre-treated with delphinidin displayed a lower incidence of mutation induced with cyclophosphamide.

The flavonoids isolated from *Solanum melongena* L. expressed their potent antioxidant activity against chromosomal aberrations induced by doxorubicin (Sudheesh *et al*., 1999; Sadilova *et al*., 2006). Kwon *et al.* (2008) indicated that phenolic-enriched extracts of *Solanum melongena* L. with moderate free radical scavenging-linked antioxidant activity had high α-glucosidase inhibitory activity and

in specific cases moderate to high angiotensin I-converting enzyme (ACE) inhibitory activity. Inhibition of these enzymes provide a strong biochemical basis for management of type 2 diabetes by controlling glucose absorption and reducing associated hypertension, respectively. This phenolic antioxidant-enriched dietary strategy also has the potential to reduce hyperglycemia-induced pathogenesis linked to cellular oxidation stress.

3.2 Effect of Cooking on Chemical Properties of Vegetables

Cooking has complex influences on the nutrient and non-nutrient content of vegetables (Holst and Williamson, 2004; Rock *et al*., 1998). Heat treatments have deleterious effects on the micronutrient content of vegetables such as decreasing vitamin C but at the same time the bioavailability of some nutrients may increase (Burg and Fraile, 1995; Murcia, Lopez-Ayerra, Martinez-Tome, Vera, and Garcia-Carmona, 2000; Rao, Lee, Katz, and Cooley, 1981; Van het Hof *et al*. (2000a, b). Conventionally, blanching is done using boiling water. This method is simple and inexpensive, but has the highest potential of leaching water-soluble components. Gawlik-Dziki (2008) reported that boiling of fresh broccoli significantly decreased in polyphenol content. On the other hand, Chuah *et al.* (2008) showed that microwave heating and stir-frying did not affect the radical-scavenging activity, total polyphenolic content, ascorbic acid content or total carotenoid contents while their levels were partly degraded during boiling. Loss of antioxidant activity was greater in the cooked tissues with prolonged boiling time. This finding indicated that cooking of peppers by microwave heating and stir-frying is better to ensure a higher retention of the bioactive components in peppers. When boiling is unavoidable, it is recommended to use less water and less cooking time, so as to retain the optimum benefits of the bioactive compounds present in peppers during cooking.

Conventional cooking method (boiling and baking) had a small effect on ascorbic acid, total phenolic, lycopene and antioxidant activity of tomatoes while frying significantly reduced those of tomatoes (Sahlin *et al*., 2004). Chen *et al.* (2000) showed that the antioxidant activity of cultivar Black Persimmon increased during storage at 15^{o}C, while frying in oil or steaming the tomatoes led to a fall in vitamin C

content but the overall antioxidant activity increased following these thermal treatments. The production of tomato paste from fresh tomatoes is an example where both homogenization and heat treatment are used and where the availability of carotenoids is enhanced (Van het Hof *et al*., 2000a). Kidmose *et al.* (2006) found that frying (160˚C) amaranth leaves, water convolvulus leaves and sweet bell pepper increased the quantity of β-carotene compared to the raw ones. Manzocco *et al.* (1998) reported that pasteurization of tea increased the antioxidant activity, which was attributed to the formation of compounds having antioxidant activity during heat treatment. The effects of food preparations (pounding or chopping) and heat treatments (100^{o}C or 200^{o}C) increased the antioxidant activity of garlic, shallot and onion (Aunanan and Kangsadalampai, 2008). In addition, blanching of garlic, shallot and onion for 90 s most fully preserved the contents of their bioactive compounds and related antioxidant potential (Gorinstein *et al.*, 2010).

3.3 Effect of Processing on Dietary Antimutagens

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. Boiling also reduced the antimutagenicity against urethane in *Drosophila melanogaster* of bananas namely, kluinamwaa, kluikai and kluihom (Limpichaisopon, 2002) and of eggplants namely, *Lycopersicon esculentum, Solanum melongena, Solanum aculeatissimum* and *Solanum torvum* (Tanruk, 2003)

The antimutagenicity of fruits and vegetables extracts was extensively heat stable and heating surprisingly caused an increase of antimutagenic potencies in *Salmonella typhimurium* TA 98 for antimutagenicity against heterocyclic amine mutagens, namely 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) and 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), especially of broccoli, white and red cabbage (Edenharder *et al*., 1995). Turkman *et al*. (2006) studied the effects of cooking methods on chlorophyll, pheophytins and colour of selected green vegetables. As a result, chlorophyll a and b content of these six vegetables were reduced in various

extents to their derivatives depending on the type of vegetable and cooking method. From a nutritional point of view, although cooking caused a loss of chlorophylls which are known to have health effects such as antimutagenic and anticarcinogenic effects, they concluded that nutritional properties of vegetables might have been maintained since chlorophyll degradation products pheophytin a and pheophytin b also have the similar health effects.

3.4 Methods Used in Antioxidant Assays

A wide range of methods are currently used to assess antioxidant capacity (Halliwell *et al*., 1995), for example for measurement of prevention of oxidative damage to biomolecules such as lipids or DNA and methods assessing radical scavenging. Both *in vivo* and *in vitro* assays are used and all methods have their own advantages and limitations. Simple scavenging assays such as the TRAP (total reactive antioxidant potential or total radical-trapping antioxidant parameter) and the TEAC (Trolox Equivalent Antioxidant Capacity) assay have gained popularity because they enable high-throughput screening on potential antioxidant capacity. Such methods are used to assess antioxidant capacity of biological matrices such as plasma, as well as single compounds, food components or food extracts.

3.4.1 Determination of Total Phenolic Content

The total phenolic content was determined according to the method described by Swain and Hills (1959), Naczk and Shahidi (1989) and Amarowicz *et al*. (2004) with minor modification. The antioxidant activity of phenolic compounds is mainly due to their redox properties which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides (Javanmardi *et al*., 2003). Total phenolic content was estimated using the Folin-Ciocalteu colorimetric method described previously with a little modification. Briefly, the appropriate dilutions of the extracts were oxidized with Folin-Ciocalteu reagent and then the reaction was neutralized with saturated sodium carbonate. The absorbance of the resulting blue color was measured with a microplate reader at 750

nm after incubation. Quantification was done on the basic of the standard curve of gallic acid. Results were expressed as gallic acid equivalent (GAE) (Cai *et al*., 2004).

3.4.2 DPPH Assay for Free Radical Scavenging Activity

DPPH assay has been widely used to evaluate the free radical scavenging effectiveness of various antioxidant substances. The assay determines the stoichiometry for the reaction of DPPH with H-donor for individual substance or the quantity of active OH-groups in complex mixture (Roginsky and Lissi, 2005). It is based on the reaction between DPPH and Trolox. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) also known as 1,1-diphenyl-2-picrylhydrazyl or R,R-diphenyl-*â*-picrylhydrazyl, is a free radical used for assessing results in a loss of absorbance at 520 nm. This assay is used to compare the radical scavenging activity of a compound with respect to that of Trolox, a watersoluble vitamin E analogue (Pietta *et al*., 1988; van den Berg *et al*., 1999). Thus, the degree of discoloration of the solution indicates the scavenging efficiency of the added substance (Fukumoto and Mazza, 2000).

3.4.3 Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay is quick and simple to perform, and reaction is reproducible and linearly related to the molar concentration of the antioxidant(s) present (Benzie *et al*., 1999). It was carried out accordingly to the method of Benzie and Strain (1996) with slight modification. The method is based on the reduction of a ferric 2, 4, 6-tripyridyl-s-triazine complex (Fe^{3+}-TPTZ) to the ferrous form (Fe^{2+}-TPTZ). The reaction is nonspecific and any half-reaction which has a less-positive redox potential under reaction condition than the Fe^{3+}/Fe^{2+}-TPTZ half-reaction will drive Fe^{3+}-TPTZ reduction. Test conditions favor reduction of the complex and thereby, color development provided that a reductant (antioxidant) is present. Ferrozine (Stookey, 1970), a compound closely related to TPTZ, has been widely used with excess ascorbic acid to measure iron. In the FRAP assay, excess Fe^{3+} is used and the rate-limiting factor of Fe^{2+}-TPTZ and hence color formation is the reduction ability of the sample.

3.5 Somatic Mutation and Recombination Test (SMART)

The Somatic Mutation and Recombination Test (SMART) in *Drosophila melanogaster* has been designed to detect genetic damage in a rapid and inexpensive way. It is an *in vivo* system that uses a eukaryotic organism with metabolic machinery similar to that found in mammalian cells (Vogel and Zijlstra, 1987). Several advantages of *Drosophila melanogaster* as a test organism for detection of chemicals with genotoxic activity have been enumerated. The main points are: a short life cycle (10 days at 25°C); easy to detect genetically controlled morphological characters; large numbers of mutants and genetically characterized strains are available; culture media are inexpensive and allow the breeding of large numbers of animals using simple facilities. Also, it is capable of activating enzymatically promutagens and procarcinogens *in vivo* (Sarikaya and Cakir, 2005).

This assay is based on induced loss of heterozygosity, which may occur through various mechanisms, such as point mutations, deletions, certain types of chromosome aberrations as well as mitotic recombination and gene conversion (Graf *et al*., 1984). It is based on the treatment of larvae during the embryogenesis, the imaginal disc cells proliferate mitotically and many genetic events such as point mutations, deletions, somatic recombination and non-disjunction can be determined on the wing of adult flies (Würgler and Vogel, 1986). If a genetic alteration occurs in one cell of the imaginal disc during mitotic proliferation, it will form a clone of mutant cells expressing the phenotype regulated by the specific genetic markers. The use of improved high-bioactivation (HB) strains of *Drosophila melanogaster*, which are characterized by increased cytochrome P450-dependent bioactivation capacity, facilitates the detection of promutagens and procarcinogens of different chemical classes (Graf and Singer, 1989; Graf and Van Schaik, 1992). The SMART assay is also well suited to determine the antimutagenicity of pure chemicals or mixtures (Negishi *et al*., 1989; Graf *et al*., 1989).

3.5.1 Wing Spot Test in *Drosophila*

The wing spot test makes use of the recessive markers multiple wing hair (*mwh*) and flare (flr^3) which alter the phenotypic expression of the hairs on the wing blade (Graf *et al*., 1984 and 1989; Szabad *et al*., 1983). The two wing hair markers are

both located on the left arm of chromosome 3 (Graf and Würgler, 1986). The appearance of multiple wing hairs (*mwh*, 3-0.0) is a recessive, homozygously viable mutation and produces multiple trichomes per cell instead of the normally unique trichome. The second marker, flare (flr^3, 3-39.0) is a recessive mutation that produces malformed wing hairs that have the shape of a flare. All three mutant alleles of *flr* are recessive zygotic lethal. However, homozygous cells in the wing imaginal discs are viable and lead to mutant wing cells. The flr^3 allele is kept over a balancer chromosome carrying multiple inversions and a dominant marker that is a homozygous lethal ($flr^3/TM3, Bd^s$: Third Multiple 3, Beaded-Serrate).

In all the experimental series analyzed, the occurrence of the various types of spots was as follows: most frequent were single spots expressing the *mwh* phenotype, less frequent twin spots with both a recombination sub-clone, and quite rare single spots with the flr^3 phenotype (Lindsley and Zimm, 1992). Different types of wing hair mutations are shown in Figure 3.1

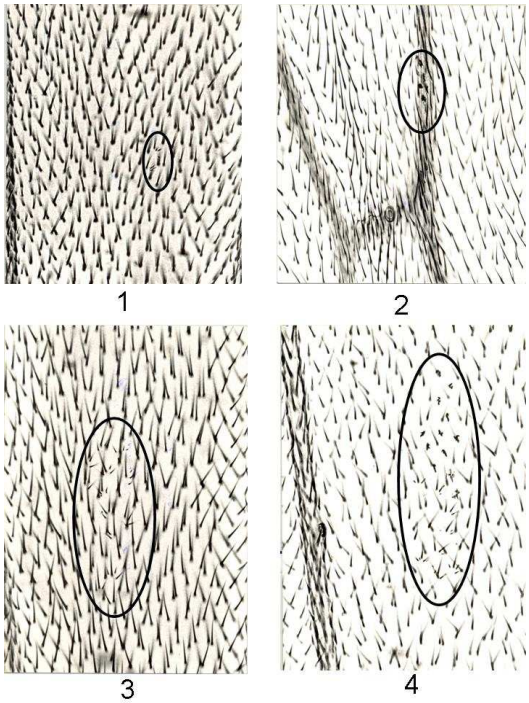

Figure 3.1 Difference types of wing hair mutation, 1) small single spots of *mwh* on wing, 2) large single spots of flare on wing, 3) large single spots of *mwh* on wing, 4) twin spots (By courtesy of Assoc. Prof. Kaew Kangsadalampai).

Several mechanisms lead to genetically marked clones (Figure 3.2). An important possibility is a mitotic recombination event between two non-sister chromatids. Twin spots are expected if recombination occurs between *flr*^{3} and the centromere (Becker, 1976). A recombination event between *mwh* and *flr*^{3} may result in a *mwh* single spot. If both types of recombination events (one between *flr*^{3} and the centromere, a second between *mwh* and *flr*^{3}) take place within the same cell, a *flr*^{3} single spot may result. Nondisjunctional or other loses of the chromosomes carrying the wild type allele represents another mechanism that may lead to single spots. Mitotic recombination in the chromosome section between the centromere (spindle fiber attachment site) and the marker *flr*^{3} leads to two daughter cells, one homozygous for *mwh*, the other homozygous for *flr*^{3}. Clonal expansion to these two cells will be recognizable on the wing blade from the two multicellular adjacent clones, one exhibiting the *mwh* phenotype (multiple hairs), the other the *flr*^{3} phenotype (misshape hairs). On the other hand, the origin of "single spot", showing either the *mwh* or the *flr*^{3} phenotype (mainly of the *mwh* phenotype, rarely also of the *flr*^{3} phenotype), cannot be clearly determined. Multiple wing hairs single spots may result from a recombination event occurring in the chromosome segment between the two marker genes. In addition, a gene mutation or deletion of the *mwh*$^+$ gene will result in a *mwh* single spot. An *flr*^{3} single spot may either result from a gene mutation or deletion of the *flr*^{3} gene, or from a rare double recombination with one recombination event to the left, and the other event to the right of the *flr*^{3} locus (Würgler *et al*., 1991).

3.5.2 Standard Mutagens for Mutagenicity of SMART (Urethane)

Urethane, a byproduct of fermentation found in alcoholic beverages, is carcinogenic in rodent and is classified by International Agency for Research on cancer as a possible human carcinogen. Urethane ($NH_2COOCH_2CH_3$), also khown as ethyl carbamate, is the ethyl ester of carbamic acid (NH_2COOH). Urethane may occur as a colorless, odorless crystal or a white, granular powder. It is slightly soluble in olive oil and soluble in water, ethane, ether, glycerol, chloroform, and ethyl ether. In the 1940s, urethane was used as a hypnotic in man at doses of 1 g/person/day and as an anesthetic for laboratory animals. In 1943, it was discovered that urethane had a carcinogenic effect in animals (Berenblum, 1982). Since 1948 it has been known that urethane is

mutagenic in *Drosophila melanogaster*. Urethane is generally used as positive standard toxicants in evaluation genotoxicity of the unknown compounds in SMART (Abraham and Graf, 1996). The major source of human exposure to urethane is from fermented food products (bread, yogurt and cheese) and alcoholic beverages (white wine and beer) (Canas *et al*., 1989; Ough, 1976). These chemical require metabolic activation to express their mutagenic activity (Frölich and Würgler, 1990b).

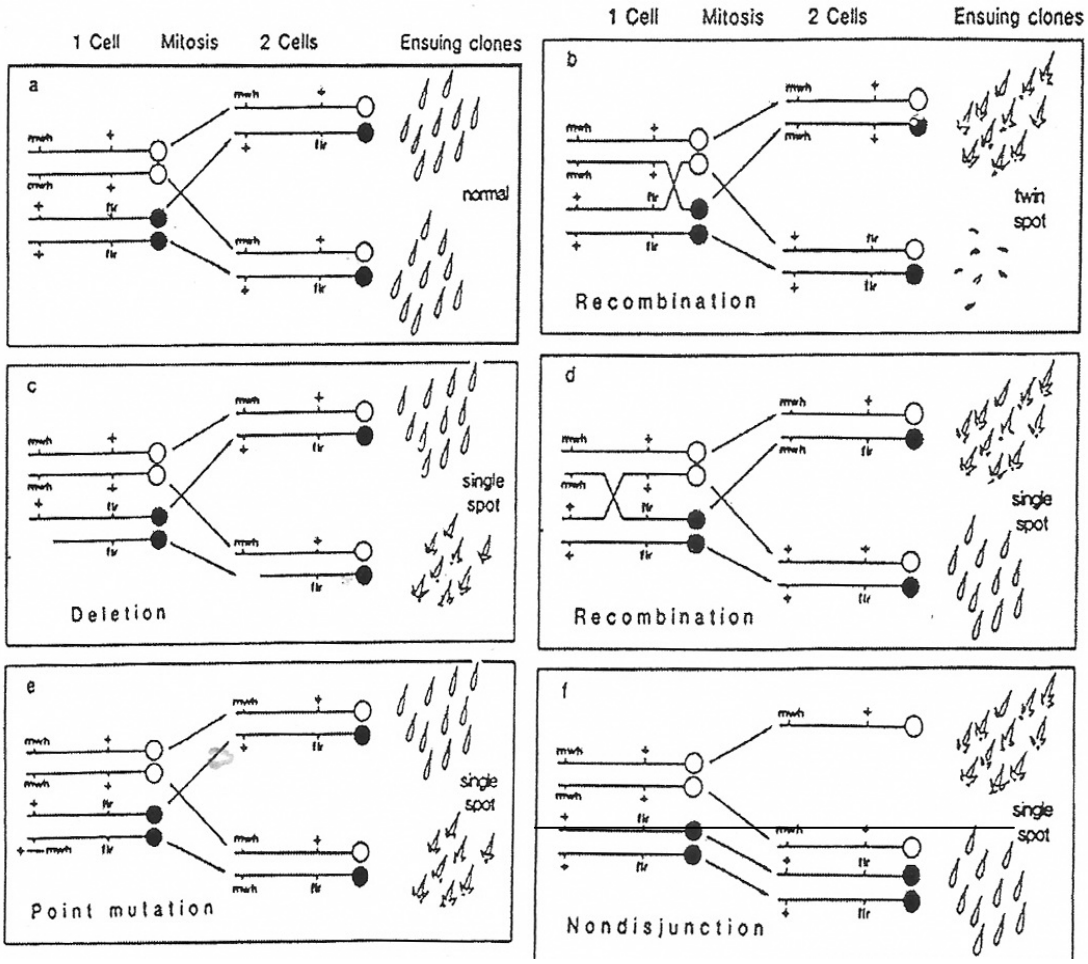

Figure 3.2 Genetics schemes illustrating various ways of spot formation in the somatic mutation and recombination test with the wing cell markers multiple wing hair (*mwh*) and flare (*flr*^{3}) (a). Twin spots are obtained by recombination proximal to the *flr*^{3} marker (b), while more distal recombination produces *mwh* single spots only (d). Deficiencies (c), point mutations (e) and nondisjunction events (f) give rise to *mwh* single spots or in analogous ways to *flr*^{3} single spots (Graf *et al*., 1984).

3.5.3 Mutagenicity of Urethane

Urethane is classified as possibly carcinogenic to humans (group 2B) by the International Agency for Research on Cancer, but evidence of urethane carcinogenicity in humans is considered inadequate (IARC, 1974). Many reports were published concerning the mutagenicity of urethane in a wide range of organism (Field and Lang, 1988). In tests with eukaryotic cells, positive and negative findings were about equal in frequency. It seemed that positive results were obtained only under conditions of appropriate metabolic activation. Urethane was genotoxic in the somatic mutation and recombination test in *Drosophila melanogaster* (number and shape of wing hairs after treatment of larvae), in a standard strain and in a strain in which genetic control of cytochrome P-450-dependent enzyme systems were altered (constitutively increased P-450 enzyme activities) (Frölich and Würgler, 1988; Frölich and Würgler, 1990a). The effects were dose-dependent and the modified strain was more sensitive to urethane by about one order of magnitude than the standard strain. This further suggested that the P-450 enzyme system involved in the activation of urethane. The frequencies of spots per wing in high bioactivation cross were higher than that in standard cross (Frölich and Würgler, 1990a). This might result from the constitutive expression of the enzyme required for the transformation of urethane into ultimate genotoxic metabolites. In addition, the urethane gas induced a significant increase of X-linked recessive lethal mutation in the germ cells of *Drosophila melanogaster* (Nomura and Kurokawa, 1997).

Urethane was found to induce point mutation, gene conversion, intrachromosomal recombination, chromosomal aberrations and sister chromatid exchanges in yeast, plant systems and mammalian cells (Schlatter and Luitz, 1990). Urethane exerts its carcinogenic effect following bioactivation to vinyl carbamate epoxide which forms RNA and DNA adducts and initiates tumorigenesis (Dahl *et al*., 1978; Leithauser *et al*., 1990). The activation of urethane is important in exerting its carcinogenic effect. The two step oxidation of urethane to the active vinyl carbamate epoxide is catalyzed primarily by cytochrome P-450 subtype 2E1 (Guengerich *et al*., 1991).

Urethane is metabolized by two different pathways (Figure 3.3). The major pathway, which accounts in rodents for over 90%, is the hydrolysis of urethane by

microsomal esterase and amidases to ethanol, ammonia and carbon dioxide (Mirvish, 1968; Park *et al*., 1993). This major pathway is probably one for detoxification. The minor pathway involves the oxidation of urethane via cytochrome P-450IIE1 (CYP2E1) to 2-hydroxyethyl carbamate, to *N*- hydroxyethyl carbamate and to vinyl carbamate, which is in turn converted by epoxidation to the putative ultimate carcinogen vinyl carbamate epoxide (Guengerich and Kim, 1991; Guengerich *et al*., 1991; Miller and Miller, 1983; Miller *et al*., 1983). Vinyl carbamate epoxide is a major strong ultimate reactive electrophilic, mutagenic and carcinogenic metabolite of urethane and vinyl carbamate in mouse (Park *et al*., 1993). Generation of the electrophilic vinyl carbamate epoxide leads to the formation of RNA and DNA adducts and the initiation of tumorigenesis (Leithauser *et al*., 1990). The schematic structures of urethane and its metabolites shown in Figure 3.4

3.5.4 Modification the Mutagenicity of Urethane

The major detoxification pathway of urethane is through hydrolysis to ethanol, ammonia and carbon dioxide (IARC, 1974), a reaction mediated by CYP2E1 (Hoffler *et al*., 2003). In rats, mice and humans, CYP2E1 is induced five to 20-fold by ethanol (Lieber, 1988 and 1990; Kurata *et al*., 1991a; Ingelman-Sundberg *et al*., 1993) which suggests that chronic ethanol exposure could increase the oxidation of urethane to its epoxide derivative. On the other hand, ethanol has been reported to decrease the metabolism of urethane, presumably by acting as a competitive substrate (Waddell *et al*., 1987; Yamamoto *et al*., 1988; Kurata *et al*., 1991b). Kristiansen *et al*. (1990) exposed adult female A/Ph mice to 0, 200, 500 or 1000 ppm urethane simultaneously with 0, 5, 10 or 20% ethanol in drinking water for 12 weeks. All mice treated with urethane developed lung adenomas with a dose-dependent manner. In addition, Mirvish (1968) reported that degradation of urethane was inhibited up to 90% by blocking esterase activity which indicated that ethanol might be formed in near equimolar amounts to the administered urethane dose. It was also shown whether the ethanol thus formed and modulated the further metabolism of urethane. Kurata *et al*. (1991a) demonstrated that acetone was a very potent, acute inhibitor of the *in vivo* metabolism of urethane when metabolically derived from 2-propanol. Conversely, pretreatment using acetone for 24 and 48 h before urethane administration accelerated

the clearance of urethane, indicating that enzyme metabolizing urethane was induced by acetone.

Hamss *et al*. (2003) investigated the modulating action of bell pepper (*Capsicum annuum*) and black pepper (*Piper nigrum*) in combination with the alkylating agent methyl methanesulfonate (MMS) and the promutagen agent ethyl carbamate (EC), against somatic mutation and recombination induced by urethane in the wing spot test using *Drosophila melanogaster*. Bell pepper was effective in reducing the mutational events induced by EC and MMS and black pepper was only effective against EC. Pretreatment of 2-day-old larvae with the spices for 24 h followed by a treatment with EC and MMS was only effective in reducing the mutations induced by EC. Suppression of metabolic activation or interaction with the active groups of mutagens could be mechanisms by which the spices exert their antimutagenic action.

Kemper *et al*. (1995) investigated the role of glutathione in protection against vinyl carbamate epoxide-mediated adduct formation and the involvement of glutathione-*S*-transferase (GST) in detoxification of vinyl carbamate epoxide. They reported that glutathione inhibited formation of ethenoadenosine in a concentration-dependent manner ranging from 1 to 8 mM. This effect was significantly enhanced by addition of rat liver GST. Investigation on the change in GST activity in relation to the observed *in vivo* antigenotoxicity of fresh vegetables, spices, tea and coffee was done by Abraham *et al*. (1998). This experiment showed that treatment with urethane alone resulted in inhibition of GST activity. Co-administration of urethane with extracts of vegetables, coffee and spices resulted in dosed-related attenuation of the inhibitory effect of urethane on GST activity. However, tea had no effect on inhibition of GST activity by urethane. Hence, and association between antigenotoxicity and GST activity could not be established. Furthermore, Abraham and Graf (1996) investigated the protective effects of coffee against somatic mutation and mitotic recombination induced by urethane were evaluated in the standard (ST) and high bioactivation (HB) crosses of the wing spot test in *Drosophila melanogaster*. The results showed high sensitivity of the HB cross to urethane. Co-administration of instant coffee was effective exerting significant dose-related inhibitory effects on the genotoxicity of urethane in the ST and genetically susceptible HB cross. Pretreatment of 2-day-old HB

larvae with coffee for 24 h followed by treatment with urethane was also effective insignificantly reducing the induction of mutation and recombination. The magnitude of the protective effects of coffee against the genotoxin (urethane) was independent of the genotype of the larvae used for treatment.

A dose-dependent increase in the genotoxic activity of urethane was observed in SMART (Frölich and Würgler, 1990b). The frequency of induction of mutation in the modification strain with increased cytochrome P450 enzyme activities was increased by about one order of magnitude compared with the standard strain. The frequencies of spots per wing in high bioactivation cross were higher than those of standard cross (Frölich and Würgler, 1990a). This might result from the constitutive expression of the enzymes required for the transformation of urethane into ultimate genotoxic metabolites.

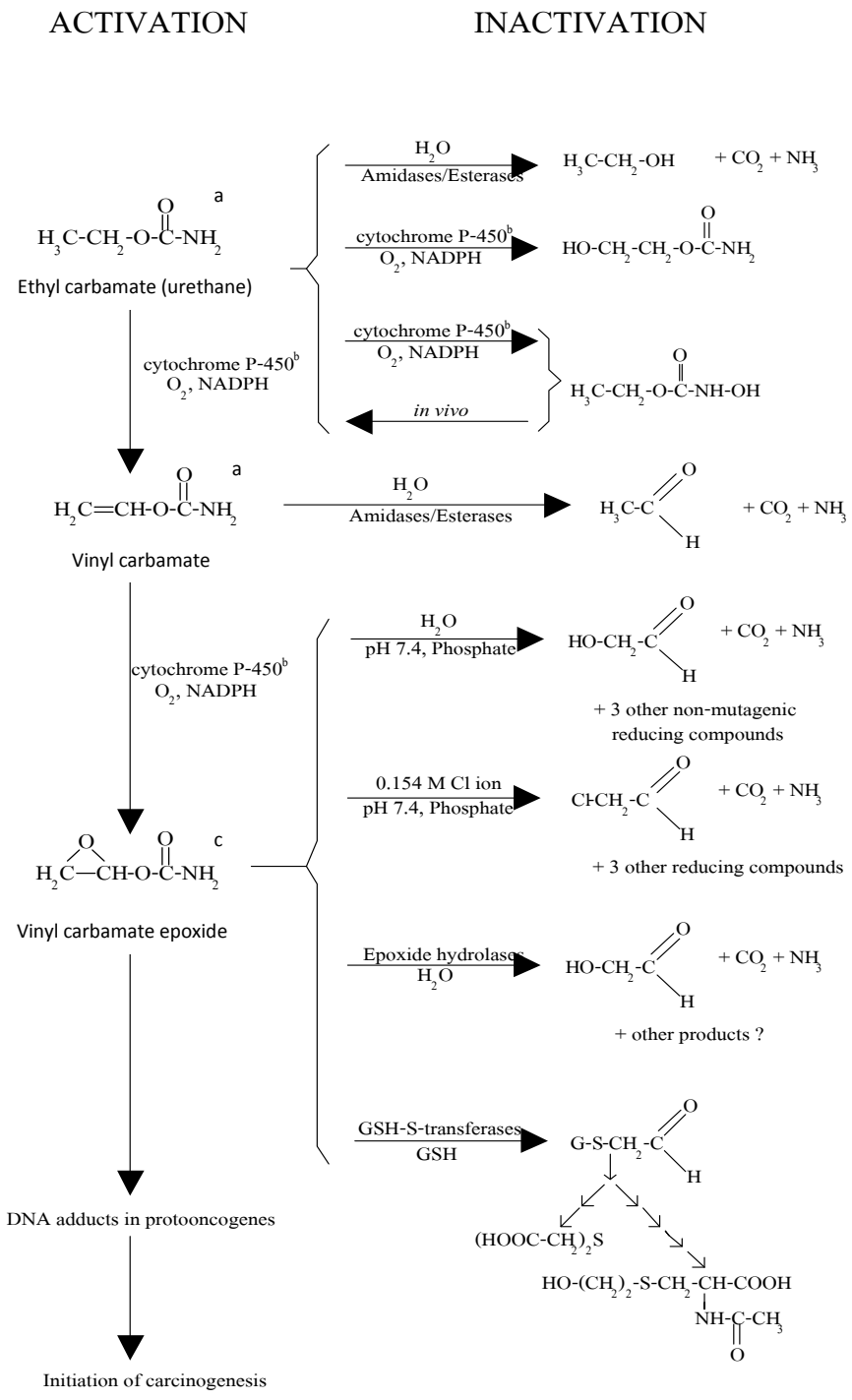

Figure 3.3 Known and probable activation and inactivation pathways of metabolism of urethane (ethyl carbamate), vinyl carbamate and vinyl carbamate epoxide. (a) Mouse liver microsomes + ethyl carbamate or vinyl carbamate + adenosine 1, N^6-ethenoadenosine. (b) Human liver microsomal cytochrome P-450 IIE1. (c) Vinyl carbamate epoxide + adenosine 1, N^6-ethenoadenosine. GSH=glutathione (Park *et al.*, 1993).

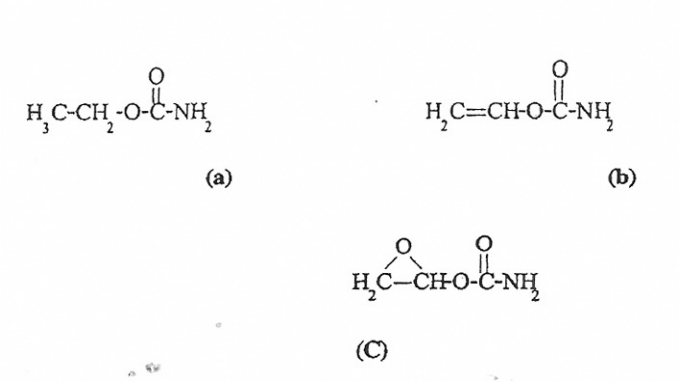

Figure 3.4 Schematic structures of urethane and its metabolites (a) Urethane (ethyl carbamate)**;** (b) Vinyl carbamate**;** (c) vinyl carbamate epoxide (Park *et al*., 1993).