CHAPTER II

LITERATURE REVIEW

2.1 Health Aspects of Plants

Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years, and have served human well as valuable components of seasoning, beverages, cosmetic, dyes, and medicines (Craig, 1999). The World Health Organization estimated that about 80% of the earth's inhabitants relied on traditional medicine for their primary health care, and most of the therapies involve the use of plant extracts or their active components. Furthermore, many Western drugs had their origin in a plant extracts. Reserpine, which is widely used for the treatment of high blood pressure, was originally extracted from the plant *Rauwolfia serpentine*, whereas digitalis, used as a heart stimulant, was derived from the foxglove plant (*Digitalis purpurea*), The Chinese herb ephedra (*Ma huang*), which contains the active substance ephedrine, was used early for the treatment of asthma, whereas salicylic acid (a precursor of aspirin) was obtained from willow tree bark (*Salix alba*) to help relieve fevers (Bruneton, 1995). Paclitaxel (TAXOL; Bristol-Myers Squibb, Princeton, NJ), a new chemotherapy agent discovered by the National Cancer Institute screening program, was obtained from the bark of the Pacific yew (*Taxas brevifolia*) as well as the needles of some other yew species, Patients with metastasis breast cancer, advanced lung cancer, cancers of the head and neck, melanoma, ovarian cancer, and lymphomas have responded positively to Taxol (Cragg *et al.*, 1993).

Many types of compounds found in plants may be responsible for the health benefits attributed to functional foods. Allyl compounds, such as those found in garlic and related foods, have been used in various parts of the world not only for aroma and flavor but also as antimicrobials, insect repellants, and modifiers of the risks of cancer and heart disease (Milner, 2000). Plants contain a variety of antioxidants (including Vitamin C, vitamin E and the carotenoids) that can inhibit oxidation of LDL cholesterol. Recently, licorice extract and the isoflavan glabridin, a major polyphenolic compound

found in licorice, were shown to markedly inhibit LDL oxidation via a mechanism involving scavenging of free radicals (Fuhraman *et al.*, 1997). Over 4,000 flavonoids, which are polyphenolic compounds found ubiquitously in foods of plants origin, have been described and categorized into flavonols, flavones, catechins, flavanones, antocyanidins, and isoflavonoids (Milner, 2000). Flavonoids have extensive biological properties that promote human health and help reduce the risk of diseases. Flavonoids extent the activity of vitamin C, act as antioxidants protect LDL cholesterol from oxidation, inhibit platelet aggregation, and act as anti-inflammatory and antitumor agents (Manach *et al.*, 1996; Cook and Samman, 1996; Hollman, 1997).

Herbs with anticancer activity

Herbs have been used as food and for medicinal purposes for centuries. In herbal medicine the term *herbs* is used loosely to refer not only to herbaceous plants but also to bark; roots; leaves; seeds; flowers and fruits of trees, shrubs, and woody vines; and extracts of the same that are valued for their savory, aromatic, or medicinal qualities. The botanical term herb refers to seed- producing plants with nonwoody stems that die down at the end of growing season. In different herbs, a wide variety of active phytochemicals, including the flavonoids, terpenoids, lignans, sulfides, polyphenolics, carotenoids, coumarin, saponins, plant sterols, curcumins, and phthalides have been identified (Craig, 1999). Several of these phytochemicals either inhibit nitrosation or the formation of DNA adducts or stimulates the activity of protective enzymes such as the Phase II enzyme glutathione transferase. Some of the more popular herbs in use today include *Echinacea*, garlic, ginseng, goldenseal, ginkgo, saw palmetto, aloe vera, and feverfew. For example, garlic (*Allium sativum* L.) is known to have antitumor properties, owing to its content of a wide variety of organic sulfides and polysulfides. (Lau *et al.*, 1986; Dauusch and Nixon, 1990; Lau, Tadi, and Tosk, 1990).

The National Cancer Institute has identified a host of cancer chemoprotective phytochemicals in some herbs. These herbs include members of the *Allium* sp. (garlic. onions, chives); members of the Labiate (mint) family (basil, mints, oregano, rosemary,

sage, and thyme): members of the Zingiberaceae family (turmeric and ginger); licorice root; green tea; flax; members of the *Umbelliferae* (carrot) family (anise, caraway, celery, chervil, cilantro, coriander, cumin, dill, fennel, and parsley); and tarragon (Carragay, 1992). In addition, many herbs contain a variety of phytosterols, triterpenenes, flavonoides, saponins and carotenoids, which have been shown from studies of legumes, fruit, and vegetables to be cancer chemoprotective (Steinmetz and Potter, 1991). These beneficial substance act as antioxidants and electrophile scavengers, stimulate the immune system, inhibit nitrosation and formation of DNA adducts with carcinogens, inhibit hormonal actions and metabolic pathways associated with the development of cancer, and induce phase I or II detoxification enzymes (Carragay 1992; Cuvelier, Berset, and Richard, 1994). Several phytochemicals inhibit tumor formation by stimulating the protective phase II enzyme, glutathione-*S*-transferase (GST). GST is a detoxifying enzyme that catalyzes the reaction of glutathione with electrophiles to form compounds that are less toxic, more water-soluble, and can be excreted easily. Examples of phytochemicals that stimulate glutathione-*S*-transferases activity include phthalides, found in umberlliferous herbs; sulfides, found in garlic and onions, curcumin in turmeric and ginger; and terpenoids, ie, limonene, geraniol, menthol, and carvone found in commonly used herbs (Steinmetz and Potter, 1991; Craig, 1999).

Garlic is reported to enhance immune function by stimulating lymphocytes and macrophages to destroy cancer cells; garlic is also reported to disrupt the metabolism of tumor cells (Dauusch, and Nixon, 1990). Garlic can inhibit the formation of nitrosamines, which are potent carcinogens, and can also inhibit the formation of DNA adducts (Milner, 1996).

Turmeric (*Curcuma longa*) imparts a rich yellow color to food. Its active phenolic constituents inhibit cancer and also have antimutagenic activity (Nagabhushan, and Bhide, 1992).Its activity is largely due to the antioxidant curcumin (a diferuloymethane), which has been shown to be an effective anti-inflammatory agent in humans (Chan and Fong, 1994).

Polyphenolics in green tea (*Camellia sinensis*) are known to possess antimutagenic and anticancer activity (Dreosti, 1996). The tumor incidence and

average tumor yield in rats with chemically induced colon cancer were significantly reduced when the rats received (-)-epigallo-catechin gallate, a major polyphenolic constituent of green tea (Kim *et al.*, 1994). In a study conducted at the New Jersey Medical School, extracts of both black and green tea significantly inhibited leukemia and liver tumor cells from synthesizing DNA (Lea *et al.*, 1993).

Lentinan, a β-glucan found in shiitake mushrooms (*Lentinus edodes*), has been shown to have antitumor activity; it was active against lung carcinoma and 2 human melanomas (Ladanyi, Timar, and Lapis, 1993). Lentinan increased production and activity of natural killer cells and macrophages, which destroy tumor cells (Craig, 1999; Mizuno, 1995).

The list of selected phytochemicals associated with cancer prevention was shown in Table 1.

Table 1 Selected phytochemicals associated with cancer prevention (Greenwald, Clifford, and Milner, 2001)

Phytochemical class	Typical compound	Food sources	Cancer prevention-related activities
Carotenoids	β-carotene, α-carotene, lycopene, lutein, astaxanthin, β-cryptoxanthin	Yellow-red and dark green vegetables and fruits	Antioxidant activity, modulation of carcinogen metabolism, inhibition of cell proliferation/oncogene expression, beneficial effect on immune function/cell transformation and differentiation, enhance cell to cell communication
Organosulphur compounds	Diallyl sulphide, allyl methyl trisulphide, dithiolthiones	Sulphides: garlic, onion, dithiolthiones: broccoli, cabbage	Increase phase II enzyme, inhibit cell proliferation, induce cell differentiation, alter steroid hormone metabolism, inhibit ornithine decarboxylase activity
Polyphenols	Phenolic acids, hydroxycinnamic acids, flavanols, flavanones, catechin	Vegetables and fruits, green tea, black tea red wine	Reduce carcinogen-DNA adduct formation, inhibit cell proliferation, induce cell cycle arrest and apoptosis, inhibit signal transduction pathways, enhance cell to cell communication, improve immune function

Table 1 (continued)

Phytochemical class	Typical compound	Food sources	Cancer prevention-related activities
Phytoestrogens	Isoflavones, lignans	Isoflavones: soybeans, soy-based foods, lignans: vegetables, cereals	Alter estrogen metabolism, decrease tyrosine kinase activity, induce cell cycle arrest and apoptosis, induce topoismerase II-mediated DNA breakage
Glucocinolates, isothiocyanates, indole	Glucobrassicin, sulphorophane, indole-3-carbinol	Cruciferous vegetables	Increase phase II enzyme activity, induce cell cycle arrest and apoptosis, inhibit cell adhesion and invasion
Terpenes	Monoterpenes (e.g. limonene, perillyl alcohol), sesquiterpenes (e.g. farnesol)	Vegetable and fruits (e.g. citrus)	Increase phase II enzyme activity, influence cell cycle progression, induce apoptosis

2.2 Characteristic of Mushrooms

2.2.1 Button mushrooms

Button mushrooms (เห็ดกระดุม, เห็ดแชมปิญอง) namely *Agaricus bisporus* is the largest among cultivated edible mushrooms all over the world despite of its complicated process of cultivation and low yield potential or biological efficiency (Banik and Nandi, 2004) (Figure 1) *Agaricus bisporus* is common and can be found growing in clusters or rings in grazed or mown grassland and it is a cultivated variety, otherwise known as "champignon de Paris". It has a smooth white to brownish cap and a white stem and flesh. White mushrooms are also available canned – plain whole or sliced, marinated or pickled Nutrient composition of mushrooms was shown in Table 2.

Figure 1 Button mushroom

2.2.2 Shiitake mushroom

Shiitake mushroom (เห็ดหอม) (*Lentinus edodes* [Berk.] Pegler), or forest mushroom and shiang- ku (fragrant mushroom), are traditional delicacies in Japan, Korea, Taiwan and China (Yang, Lin, and Mau, 2001) (Figure 2) Shiitake, comes from the Japanese *Shii*, which means oak and *take* which means mushroom, it is the second largest cultivated mushroom species in the world, second only to the white button mushroom or champignon (*Agaricus bisporus*) (Chang, 1996, Sugui *et al.*, 2003). It grows on the trunks or stumps of trees.

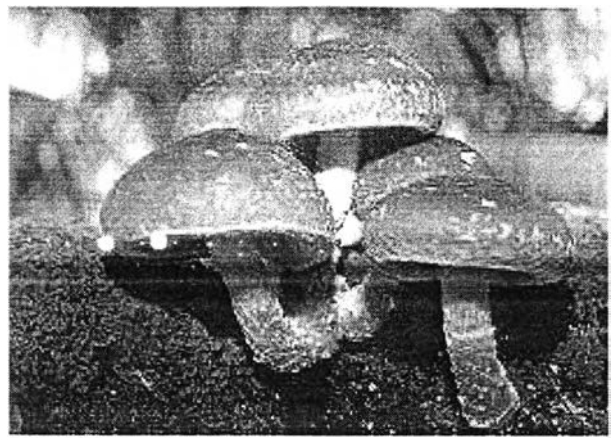


Figure 2 Shiitake mushroom

2.2.3 Pleurotus mushrooms

Two Pleurotus mushrooms, i.e. abalone and oyster mushrooms are commercially popular in Taiwan. Abalone mushroom (*Pleurotus cystidiosus* O.K. Miller) (Figure 3) is also called summer oyster mushroom. Oyster mushroom (*Pleurotus ostreatus* [Jacquin: Fries] Kummer) (Figure 4), also called hsiu- jen- ku (mini oyster mushroom), is apparently smaller and lighter than abalone mushrooms (Yang *et al*, 2001). Oyster mushroom is a wood-rotting fungus produced on lignocellulose substrates in a number of countries on a large scale for the food industry. Edible basidiomycete mushroom with a funnel-shaped cap can reach 12 cm in diameter. The stem is short and off-centre. The oyster mushroom grows from October to March on dead wood or on the trunks of deciduous trees. The shell-shaped or oyster-shaped varieties of this mushroom have white flesh, but their caps may be white, pink, yellow, grey or dark brown. The color depends on the variety and the growing conditions. Young oyster mushrooms have a cap which ranges in color from dark grey to steel blue; when mature, they turn pale brown. Under the cap are white or pale grey gills.

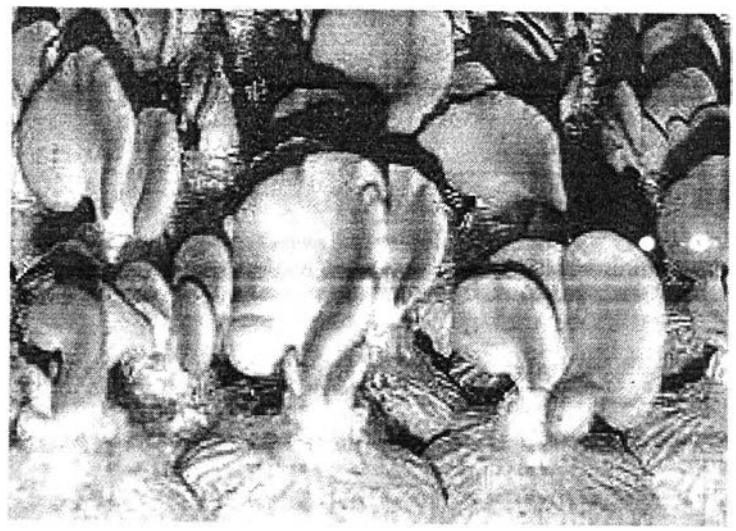


Figure 3 Abalone mushroom

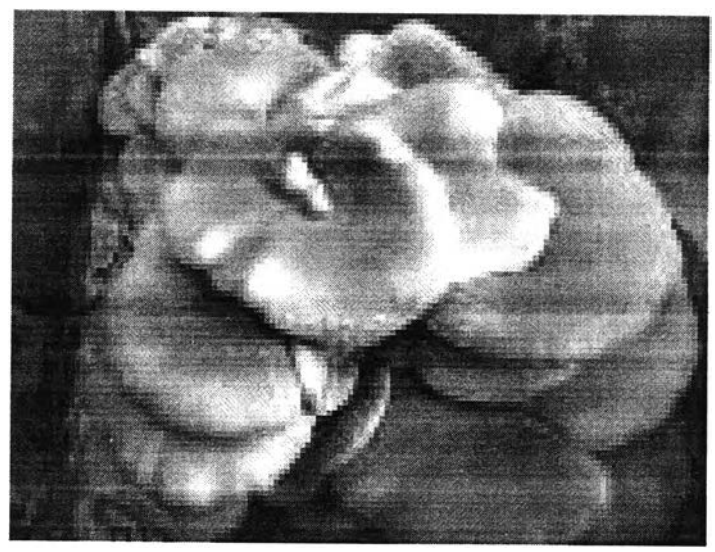


Figure 4 Oyster mushroom

Table 2 Nutrient composition of Mushrooms (composition per 100 grams edible portion)

Mushroom	Moisture	Calorie	Fat	CHO	Protein	CF	Ash	Mineral			Vitamin			
	g	kcal	g	g	g	g	g	Ca mg	Fe mg	P mg	B1 mg	B2 mg	Niacin mg	C mg
Button[a]	92.30	27	0.33	4.50	2.09	1.50	0.78	ND	ND	ND	ND	ND	ND	ND
Shiitake[b]	91.60	26.61	0.121	4.19	2.19	0.934	0.634	6.44	1.06	45.78	0.001	1.03	3.23	0
Oyster[c]	92.0	28	0.30	4.30	2.10	0.50	0.80	0.80	0.30	61	0.02	0.13	2.70	21
Abalone[c]	91.10	29	0.40	4.80	1.60	1.20	0.90	3.0	1.10	78	0.01	0.24	2.8	11

CF= Crude fiber, ND= No data

a= Mattila *et al.*, 2002

b= สุนันท์ พงษ์สามารถ และคณะ, 2529

c= กองโภชนาการ กรมอนามัย, 2530

2.3 Medicinal values of Mushrooms

2.3.1 Antimutagenicity and Antigenotoxic effect

Kim, Kacew, and Lee (1999) found that polysaccharides of some plant (*Aloe barbadensis Miller*, *Lentinus edodes, Ganoderma lucidum,* and *Coriolus versicolor*) produced both antigenotoxic and antitumor activities in *in vitro* model. Liu, Ooi, and Chang (1997) suggested that mushroom polysaccharide extracts possessed superoxide and hydroxyl radical scavenging activities. However, the mechanism of free radical scavenging by polysaccharides is still not fully understood. It is possible that the protein content of polysaccharide extracts may be directly effective in free radical scavenging activity. *Letinula edodes* has been shown to contain compounds that exert a protective effect against carcinogenesis (Chihara *et al.*, 1969). An antimutagenic effect of lentinan in combination with antineoplastic agents *in vivo* was also reported. Lentinan is not only useful for cancer treatment as an immunopotentiator in combination with anti-cancer drugs, but may also prevent the increase of chromosomal damage induced by anti-cancer drugs *in vivo* (Ladanyi, Timar, and Lapis, 1993). Lima *et al.* (2001) indicated that aqueous solutions extracted from shiitake mushroom exhibit antimutagenic activity against the in vivo DNA- damaging effect of alkylating agent. Sugui *et al.* (2003) showed that the antimutagenic effect of *L. edodes* may vary among lineages. Three of the four lineages tested, exhibited antimutagenic activity by decreasing mice bone marrow micronucleus test induced by a direct- acting alkylating agent. Gruter, Friederich, and Wurgler (1991) have demonstrated that season, geographic or intraspecies differences may influence the chemical composition of mushrooms and consequently moderate their effect. Oliveira *et al.* (2002) found that aqueous extracts of *Agaricus blazei* (sun mushroom) have an antimutagenic effect in mammalian cell *in vitro*. Menoli *et al.* (2001) also found an antimutagenic effect with a mixture of *A. blazei* lines in the micronuclei test in v79 cells treated with methyl methanesulphonate (MMS). *Agaricus bisporus* is the most widely cultivated and consumed edible mushroom (Chang, 1999). More recently, Shi, benzei, and Buswell (2002) showed that cold water extracts of *A. bisporus* fruit bodies prevented H_2O_2-induced oxidative. They reported for the first time that the genoprotective effect of *A. bisporus* is associated with a heat-labile protein, designated FIIβ-1, present in the fruit

body and which has been identified as tyrosinase. Tyrosinase is one of the major phenoloxidases which cause "browning" in fruits, vegetables and mushrooms, and is the major phenoloxidase present in *A. bisporus* (Ratcliffe *et al.*, 1994). The nature of the genoprotective activity of tyrosinase is dependent upon the two associated catalytic activities of the enzyme, namely hydroxylation of tyrosine to L-DOPA and the subsequent oxidation of L-DOPA to dopaquinone (Gerritsen, Chapelon, and Wichers, 1994). This is perhaps surprising since L-DOPA is normally associated with toxic pro-oxidant effects attributed to metabolic and autooxidative breakdown in the presence of molecular oxygen which produces highly unstable electrophilic Dopa-(semi)quinones. Redox-cycling of these compounds releases potentially harmful oxy radicals, hydrogen peroxide, semiquinones and quinones (Graham, 1978; Cohen, 1985), which have been linked to antitumour activity and to neurotoxic damage associated with L-DOPA-based treatment of Parkinson's disease.

2.3.2 Antioxidant properties

Oxidation is essential to many living organisms for the production of energy to fuel biological process. However oxygen- centered free radicals and other reactive oxygen species that are continuously produced *in vivo*, result in cell death and tissue damage. Oxidative damage caused by free radicals may be related to aging and diseases, such as atherosclerosis, cancer and cirrhosis. However, antioxidant supplements, or foods containing antioxidants, may be used to help the human body reduce oxidative damage (Yang *et al*, 2002). Traditionally, fruits, vegetables and green tea are seen as rich sources of natural low-molecular weight antioxidants including ascorbic acid, α-tocopherol, carotenoids, flavonoids and polyphenols. More recently, mushrooms have been investigated as a potential source of natural antioxidants. This special group of fungi has long been acknowledged in Eastern cultures as possessing a wide range of medicinal properties (Chang and Buswell,1996), and modern techniques have identified numerous bioactive mushroom components which are variously reported to exhibit anti-cancer, anti-tumour, anti-viral, immunomodulatory, hypocholesterolaemic and hepatoprotective activities (Shi *et al*, 2002). Benzie, Wu, and Buswell (1998) showed that several mushroom species also display antioxidant power in the Ferric Reducing Antioxidant Power (FRAP)

assay, and polysaccharoproteins from several mushroom species were reported to scavenge active oxygen species (Liu, Ooi, and Chang, 1997). A thermostable antioxidant protein isolated from the Shiitake mushroom, *L. edodes*, also suppressed the oxidative degradation of 2-deoxyribose and ΦX174 DNA by hydroxyl radical (Kawakishi and Tanigawa, 1997). Yang, Lin, and Mau (2002) showed that total phenols were the major naturally occurring antioxidant components and small amounts of α- tocopherol found in methanolic extracts from shiitake, abalone and oyster mushrooms. Overall, oyster mushrooms were better in antioxidant activity, reducing power and scavenging abilities and higher in total phenol content than other mushrooms. Cheung, Peter, and Ooi (2003) found that total phenolics of shiitake mushroom in the water extracts were higher than that of the methanol extracts.

2.3.3 Antitumor activity

Antitumoral activities of two polysaccharides, *L. edodes* mycelium (LEM), and KS-2, extracted from shiitake mushroom were also identified in rodents (Wasser and Weiss, 1999). Lentinan, a β- glucan, was the first antitumor compound isolated from shiitake mushroom (Chihara *et al.*, 1970). Although its mechanism of action is not completely clear, lentinan inhibits tumorigenesis mainly by activating the immune system and inducing gene expression of immunomodulatory cytokines and their receptors (Ooi and Liu, 2000). Lentinan increased production and activity of natural killer cells and macrophages, which destroy tumor cells (Craig, 1999; Mizuno, 1995). Some studies identified antitumoral activity of *Agaricus blazei* in rodents and showed that polysaccharide- protein complexes present in *A. blazei* are responsible for this effect (Oliveira *et al*, 2002).

2.3.4 Antibacterial activity

Recent studies of Shiitake have demonstrated its antimicrobial properties. Hirasawa *et al* (1999) found that three kinds of antibacterial substances were extracted by chloroform, ethylacetate or water from dried Shiitake mushrooms (*Lentinus edodes*). These substances possess efficient antibacterial activities against *Streptococcus* spp., *Actinomyces* spp., *Lactobacillus* spp., *Prevotella* spp., and *Porphyromonas* spp. of oral

origin. In the mycelium-free culture fluid of *Lentinus edodes* was bacteriostatic against *Streptococcus pyogenes*, *Staphylococcus aureus* and *Bacillus megaterium*. The substance responsible for the activity was heat-stable. These characteristics suggested that the component might be lenthionine, an antibacterial and antifungal sulphur-containing compound and the culture fluid was less toxic to human tissue culture cells than to microbes (Hatvani, 2001).

2.4 Mutagenicity of mushrooms

Hydrazines comprise a class of chemical carcinogens, with express their carcinogenicity following metabolism to reactive intermediates, catalysed by enzyme systems such as the cytochrome P450- dependent mixed- function oxidases and the flavin monooxygenases (Walton *et al.*, 1997a). Hydrazines occure naturally and have been encountered in relatively high amounts in a number of mushroom species such as *Gyromitra esculata* and *Agaricus bisporus*, the latter being the most commonly consumed edible mushroom (Toth, 1995). The most extensively consumed species of mushroom, *Agaricus bisporus*, contains a number of aromatic hydrazines (Figure 5), an established class of indirect- acting chemical carcinogen (Toth, 1988), among which the most abundant is agaritine, [β-N-[γ-L (+)-glutamyl]-4-(hyroxymethyl) phenylhydrazine] in high concentrations [up to 300 mg/ kg fresh weigh](Lui, *et al*, 1982; Shephard, Gunz ,and Schlatter, 1994). Lifttime administration of *Agaricus bisporus*, raw or baked, to mice for 3 days a week followed by a balanced semisynthetic diet for remaining days, induced tumors at digestive tract, liver and lungs (Toth and Erickson, 1986; Toth *et al*, 1997). Moreover, ethanolic and aqueous extracts of the mushroom exhibited direct- acting mutagenicity in the Ames test (Wright *et al.*, 1982) and agaritine was weakly mutagenic, in the absence of an activation system, in *Salmonella typhimurium* strain TA104, TA 2637 (Sterner *et al*, 1982; Walton *et al*, 1997a; Walton *et al*, 1997b). The content of agaritine declines with the age of the mushroom. It is reduced after storage of the freshly harvested mushrooms and after cooking (Ross, Nagel, and Toth 1982). Walton, Walker, and Ioannides (1998) found that mutagenic activity was remained even following baking of the mushroom at 225^{o} C for 10 min but more prolonged baking, for example 4 hr at 100^{o} C

reduced mutagenicity. Piilegarrd *et al* (1997) found that no carcinogenicity of a diet containing raw freeze- dried mushroom in the A/J mouse lung tumor model. Carcinogenicity studies have previously been performed with *A. bisporus* is summarized in Table 3 (Piilegarrd *et al*, 1997).

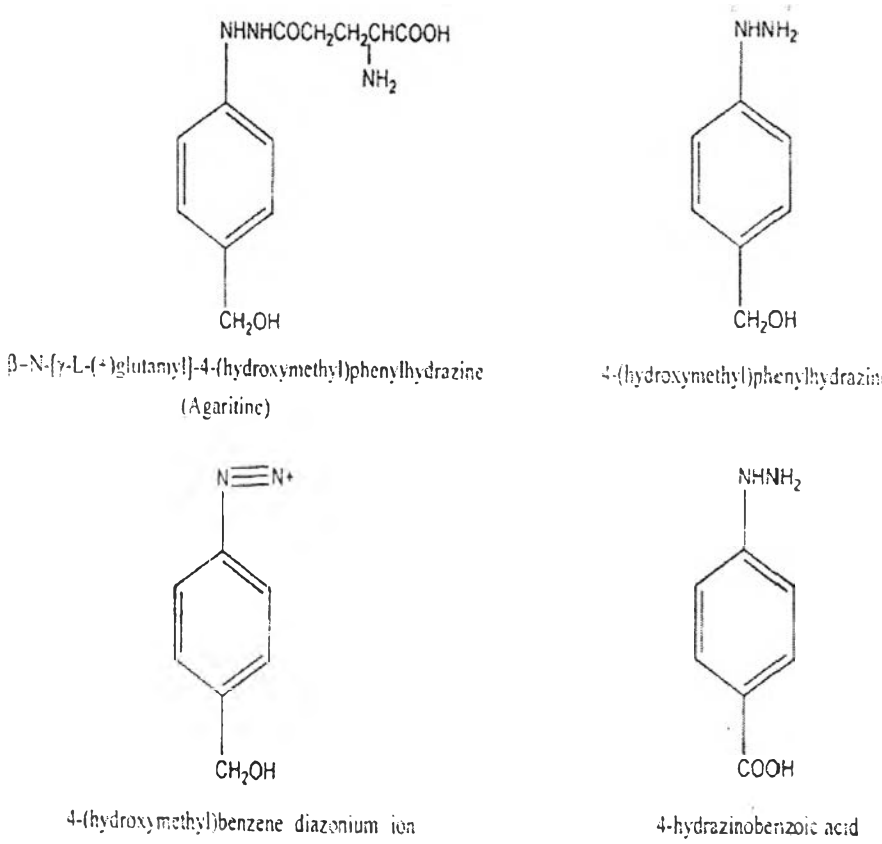


Figure 5 The structure of mushroom hydrazines

Table 3 Carcinogenic effect of *A. bisporus* or phenylhydrazine derivatives (Piilegarrd *et al.*, 1997)

Administration of substrate	Species	Duration	Carcinogen	Target organs
Raw mushroom (3 days/weeks) + semisynthetic diet (4 days/weeks) (Toth and Erickson, 1986)	Mice (M/F)	For life	Yes	Bone, forestomach, lung
30% dried mushroom (Matsumoro *et al.*, 1991)	Rat (F)	500 days	No	
25% cooked mushroom (Logten, Tenkelarr, and Esch, 1971)	Rats (M/F)	2 years	No	
0.0625%*(M/F) or 0.03125 (M) agaritine in drinking water (Toth *et al.*, 1981)	Mice (M/F)	For life	Yes	
0.0125%4(carboxy) phenylhydrazine, HCl in drinking water (McManus, Toth, and Patil, 1987)	Mice (M/F)	For life	Yes	Smooth muscle cells of aorta and larger arteries
0.0625% of the N'-acetyl derivative of 4(carboxy)phenylhydrazine, HCl in drinking water (Toth *et al.*, 1978)	Mice (M/F)	For life	Yes	Lung, blood vessel
Single dose of 400 µg 4-(hydroxymethyl)benzenediazonium tetrafluroborate (Toth and Nigel, 1982)	Mice (M/F)	For life	Yes	Glandular stomach

M= male, F= female

2.5 Food preservation

2.5.1 *Low-acid, thermally processed, packaged in hermetically sealed containers.*

Any food, other than alcoholic beverages, with a finished pH greater than 4.6 Exception:

Note: Tomatoes and tomato products having a finished equilibrium pH less than 4.7 are not classified as low- acid foods.

Thermally processed means the application of heat to food, either before or after sealing in hermetically sealed containers, for a period of time and at a temperature scientifically determined to be adequate to ensure destruction of microorganisms of public health significance (William, 2005).

Effect of thermal process on biochemical changes of vegetables

Most of the vegetables are cooked by boiling in water or microwaving before consumed. These cooking processes would bring about a number of changes in physical characteristics and chemical composition of vegetables (Rehman, Islam, and Shah, 2003; Zhang and Hamauzu, 2004). Zhang and Hamauzu pointed out that cooking affected the antioxidant components and antioxidant activity of broccoli. Ismail, Marjan, and Foong (2004) found that thermal treatment decreased the total phenolic content in all vegetables such as kale, spinach, cabbage, swamp cabbage and shallots and antioxidant activity in some of them. Turkmen, Sari, and Velioglu (2005) reported that after cooking, total antioxidant activity increased or remained unchanged depending on the type of vegetable but not type of cooking. Moreover, Padmajaprasad, Kaladhar, and Ramash (1997) found that the naturally occurring β-form of *N*-oxalyldiaminopropionic acid (β-ODAP) present in *Lathyrus sativus* is the main neurotoxic principle implicated in neurolathyrism. The α-form of ODAP has been shown to be less toxic to experimental animals. The reduction in β-ODAP toxicity using detoxification treatments was found to be a more effective and simple way of reducing seed toxicity than the isomerisation of β-ODAP to the α-isomer as observed during cooking.

2.5.2. *Acidic foods:*

2.5.2.1 Acid foods These are foods with a natural pH of 4.6 or less (most fruits).

2.5.2.2 Acidified foods These are low-acid foods to which acid(s) or acid food(s) are added; these foods include, but are not limited to: beans, cucumbers, cabbage, artichokes, cauliflower, puddings, peppers, tropical fruits and fish, singly or in any combination. They have a finished equilibrium pH of 4.6 or below. These foods may be called, or may purport to be, "pickles" or "pickled _____." Carbonated beverages, jams, jellies, preserves, acid foods (including such foods as standardized and nonstandardized food dressings and condiment sauces) that contain small amounts of low-acid food(s) and have a resultant finished 4 equilibrium pH that does not significantly differ from that of the predominant acid or acid food, and foods that are stored, distributed and retailed under refrigeration are excluded from coverage of this part (William, 2005).

2.5.3 Fermented foods

Preservation of foods by fermentation is a widely practiced and ancient technology (Caplice and Filzgerald, 1999). Fermented foods are produced through a process that increases the acid level, or lowers the pH, by taking advantage of the action of specific microorganisms. This is often referred to as a pickling process. Pickled foods should have a pH of 4.6 or less. Fermented foods may be classified into products that essentially undergo an acidic, alcoholic or enzymatic fermentation. Important fermented products include fermented milks, most cheeses, fermented sausages, fermented vegetables and vinegars. Different microorganisms are utilized in the different food products to produce several different acids. They are these acids and the byproducts produced through the metabolism of these microorganisms that produce the preservation effect (i.e., acid) and the unique flavor and texture of fermented foods. It is also the presence of the naturally made acids that makes the food safer with a pH below 4.6 (William, 2005).

Fermentation ensures not only increased shelf life and microbiological safety of a food but also may make some foods more digestible and in the case of cassava, fermentation reduces toxicity of the substrate. Lactic acid bacteria because of their unique

metabolic characteristics are involved in many fermentation processes of milk, meats, cereals and vegetables. As raw vegetables have a high microbial load and cannot be pasteurized without compromising product quality, most vegetable fermentations occur as a consequence of providing growth conditions (such as added salt) that flavor the lactic acid bacteria (Buckenhuskes, 1997). Scanchez, Palop, and Ballesteros (2000) found that 149 strains of lactic acid bacteria isolated from the spontaneous fermentation of "Almagro" eggplants and *Lactobacillus plantarum* became the predominant species during the fermentation.

2.5.3.1 Lactic acid fermented foods

Lactic acid fermentation in East-Asia has been applied for various other purposes. It is used to prepare savory acidic dishes with vegetables and cereals, to ensure the quality of rice wines, and to make beverages from plant materials. However, the most important role of lactic acid fermentation in the Asian diet is the preservation of perishable vegetables and fishes in sanitary and safe conditions. The organic acids, mainly lactic acid and acetic acid, produced by lactic acid bacteria are effective antimicrobial agents, and they reduce the pH in the foods to prevent the growth of most hazardous food microorganisms (Lee, 1997; Svanberg and Lorri, 1997).

Steinkraus (1983) established the following classifications of lactic acid fermented food products: acid fermented vegetables, acid-fermented bread and pancakes, acid-fermented cereal gruels, acid fermented seafood/rice and meat/rice mixtures, and acid-fermented milk and milk/cereal foods.

Acid fermented vegetables

Acid fermented vegetables produced in different regions of the world. The best-tasting kimchi is attained before overgrowth of *Lactobacillus brevis* and *Lactobacillus plantarum* with an optimal product pH of 4.5. The fermentation is manipulated by the salt concentration and temperature (Lee, Adler-Nissen, and Barwald, 1994).

Preservation of vegetables by kimchi fermentation

History

Kimchi is a unique traditional fermented vegetable product of Korea. Kimchi fermentation is the Korean method of preserving the fresh and crispy texture of vegetables for consumption during the winter when fresh vegetables are not available. The raw materials for kimchi are korean cabbage and radish and minor ingredients include garlic, red pepper, green onion, ginger and salt, and the optimal range of salt concentration of kimchi is 3.0-5.0% (Lee, 1997).

Physiological effects of kimchi

The possibility of nitrosamine formation during kimchi fermentation has led to extensive studies on this matter. Fresh cabbage contains large amounts of nitrate varying from 55 to 2500 ppm, but it is reduced rapidly after 1 week of kimchi fermentation. Nitrite content in kimchi ingredients is very low, ranging from 0 to 0.56 ppm, and increases slightly during the first 3-5 weeks of fermentation at 5°C and then decreases rapidly. N-Nitrosodimethylamine was not detected for the first 3-4 weeks and then appeared at a level of 0.001-0.004 ppm. It can be concluded that kimchi fermentation reduces the risk of nitrosamine formation in vegetables. The physiological effects of kimchi ingredients have been studied widely and are summarized in Table 4. Kimchi has high enough concentrations of fiber to prevent constipation and colon cancer, and the probiotic effect of lactic acid bacteria grown to 10^8/ml (Lee, 1997).

Table 4 Biologically active compounds in kimchi (Oh, Hwang, and Leitzmann, 1994)

Chemical compounds	Occurrence	Possible effect
Benzyl isothiocyanate, indole compound,thiocyanate, flavonoid	Chinese cabbage, red pepper, Allium vegetable	Antibiotic, anticarcinogenic, immune stimulant
Sitosterol	Chinese cabbage	Reducing the cholesterol level
Diallyl sulfide, dlallyl trisultide, diallylmcthyl sulfide	Allium vegetable	Anticarcinogenic, antioxidant, fibrinolytic
Gingerrol, gingerin	Ginger	Antibiotic, fibrinolytic
Capsaicin	Red pepper	Laxative, sccretion of neuropeptides
Lactic acid bacteria	kimchi	Antagonistic
Bacteriocin	kimchi	Antibiotic
L-(+)-Lactic acid	kimchi	Modulation of T-cell function
Acetylcholine	kimchi	Laxative
Dextran	kimchi	Laxative
Aminobutyric acid	kimchi	Laxative
Acetate	kimchi	Antibiotic

2.5.4. *Other foods*

This includes any food that does not meet the above criteria, (i.e., bakery items, meats, fish, poultry, etc.). The above description of the various food categories does not include products that are regulated by the USDA (i.e., meat and poultry). In each of the above classifications, federal and state agencies regulate the food under different sets of regulations. The following table illustrates the relationships between pH, water activity and current low-acid and acidified regulations. The table indicates whether a product is a 5 low-acid or an acidified food and therefore must meet the registration and process filing requirements of FDA (William, 2005).

2.6 Somatic Mutation and Recombination Test (SMART)

The somatic mutation and recombination test (SMART) for the identification of genotoxic agents in *Drosophila* have received increasing interest in the recent past, because they are rapid and sensitive test. These assays are able to detect a wide spectrum of genetic endpoints, such as point mutations, deletions, certain types of chromosome aberrations as well as mitotic recombination and gene conversion (Graf *et al.*, 1984; Vogel *et al*, 1986). This eukaryote offers the advantages of having a short generation time (approx. 10 days at 25^{o}C), needing very inexpensive culture media and allowing the breeding of large numbers of animals using simple facilities. In addition, it is well established that *Drosophila* possesses a versatile system for the metabolism of xenobiotics (Baars, 1980; Hallstrom, Blanck, and Atuma, 1984). *Drosophila* has detoxification-activating systems similar to that found in mammal cells, which makes it possible to extrapolate data to mammals (Baars, 1980; Hallstrom *et al*, 1984; Zijlstra, Vogel, and Breimer, 1984).

The use of SMART assays is based on the treatment of larvae, and besides the number of mutated spots appearing in the adult flies, indicating the frequency of genetic events, the size of the spots indicates the time of action during embryogenesis. Two different test systems have been amply explored, i.e. the wing spot test and the eye spot test. Both are based on the fact that during early embryonic development, groups of cells (imaginal discs) are set apart. They proliferate mitotically during the larval development until they differentiate during metamorphosis into structures of the body of the adult fly (eyes, wings, etc.). The somatic assays take advantage of the possibility to expose such large populations of mitotically growing cells in the imaginal discs of larvae. If a genetic alteration occurs in one of these imaginal disc cells, this alteration will be present in all the descendant cells and will form a clone of mutant cells. If the alteration causes a visible change in the phenotype, the mutant cell clone can be detected as a spot of mutant cells on the body surface of the adult flies.

The SMART assays were developed to detect the loss of heterozygosity of suitable gene markers, which determine detectable phenotypes expressed on the eyes or the wings of the flies. Owing to these advantages, the SMART assays have become a very

suitable approach for genotoxicity testing of chemical and physical agents (Graf *et al.*, 1989; Vogel and Nivard, 1993; Wurgler and Vogel, 1986).

2.6.1 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. The two wing hair markers are both located on the left arm of chromosome 3. The appearance of multiple wing hairs (*mwh*, 3-0.0) is a homozygous viable recessive mutation producing multiple trichomes per cell instead of the normally unique trichome. The second marker, flare (*flr*^{3}, 3-39.0), is a zygotic recessive lethal but homozygous cells in the wing imaginal disc survive and lead to wing blade cells with short, thickened, and misshapen trichromes. In stock cultures, the *flr*^{3} alleles have to be balanced over inverted chromosomes such as TM1 or TM3 because it is a recessive zygotic lethal.

There exist several mechanisms that lead to genetically marked clones (Figure 6). 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, 1966). 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 spots", 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 recombinational event occurring in the chromosome segment between the two marker genes. But also a gene mutation or deletion of the *mwh* gene will result in a *mwh* single spot. A *flr*^{3} single spot may either result from a gene mutation or a deletion of the *flr*^{3} gene, or from a rare double recombination with one recombinational event to the left, and the other event to the right of the *flr*^{3} locus (Wurgler, Graf, and Frolich, 1991). Different types of wing-hair mutations are shown in Figure 7.

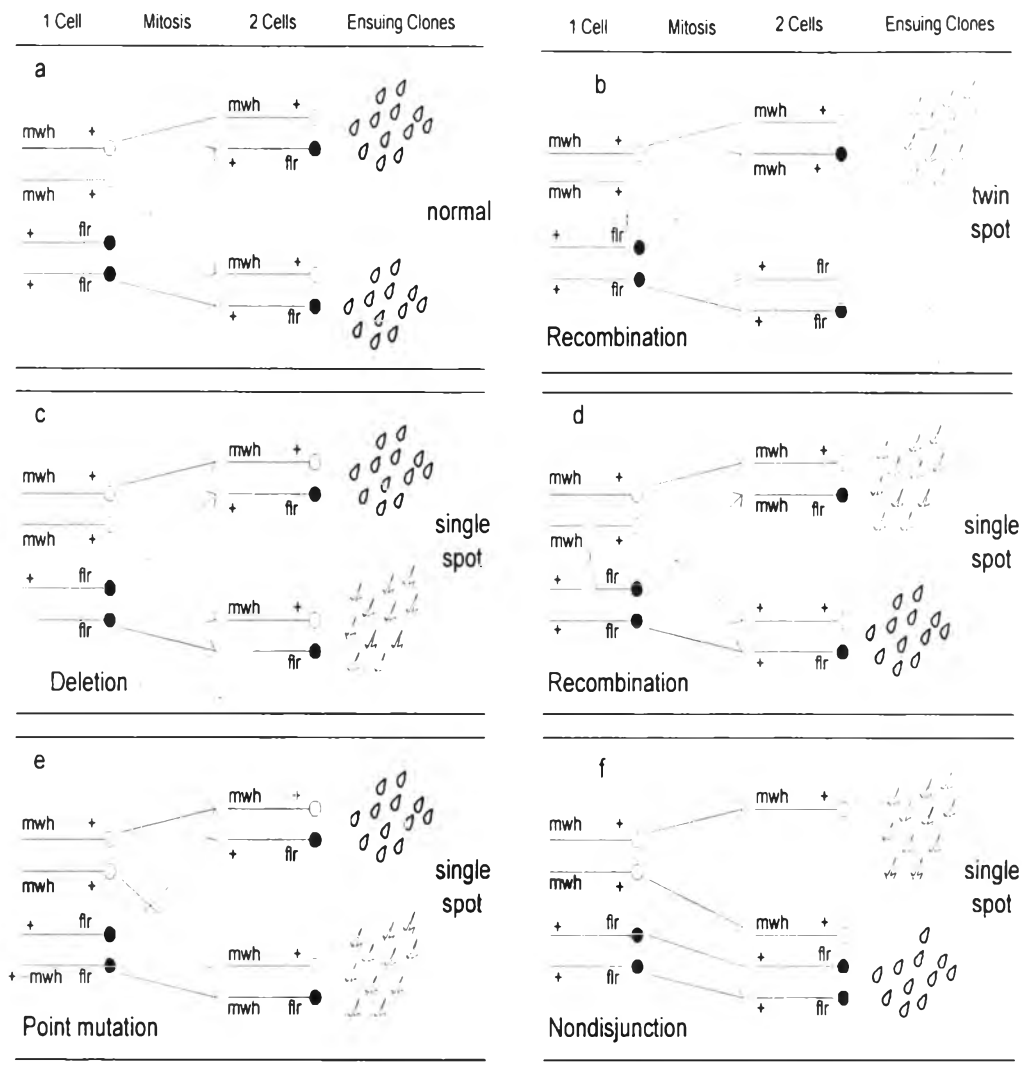


Figure 6 Genetic schemes illustrating various ways of spot formation in the somatic mutation and recombination test with the wing cell markers multiple wing hairs (*mwh*) and flare (*flr*^{3}) (a). Twin spots are obtained by recombination proximal to the *flr*^{3} marker (b), while more distal recombination 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 (not illustrated) (Graf *et al.*, 1989).

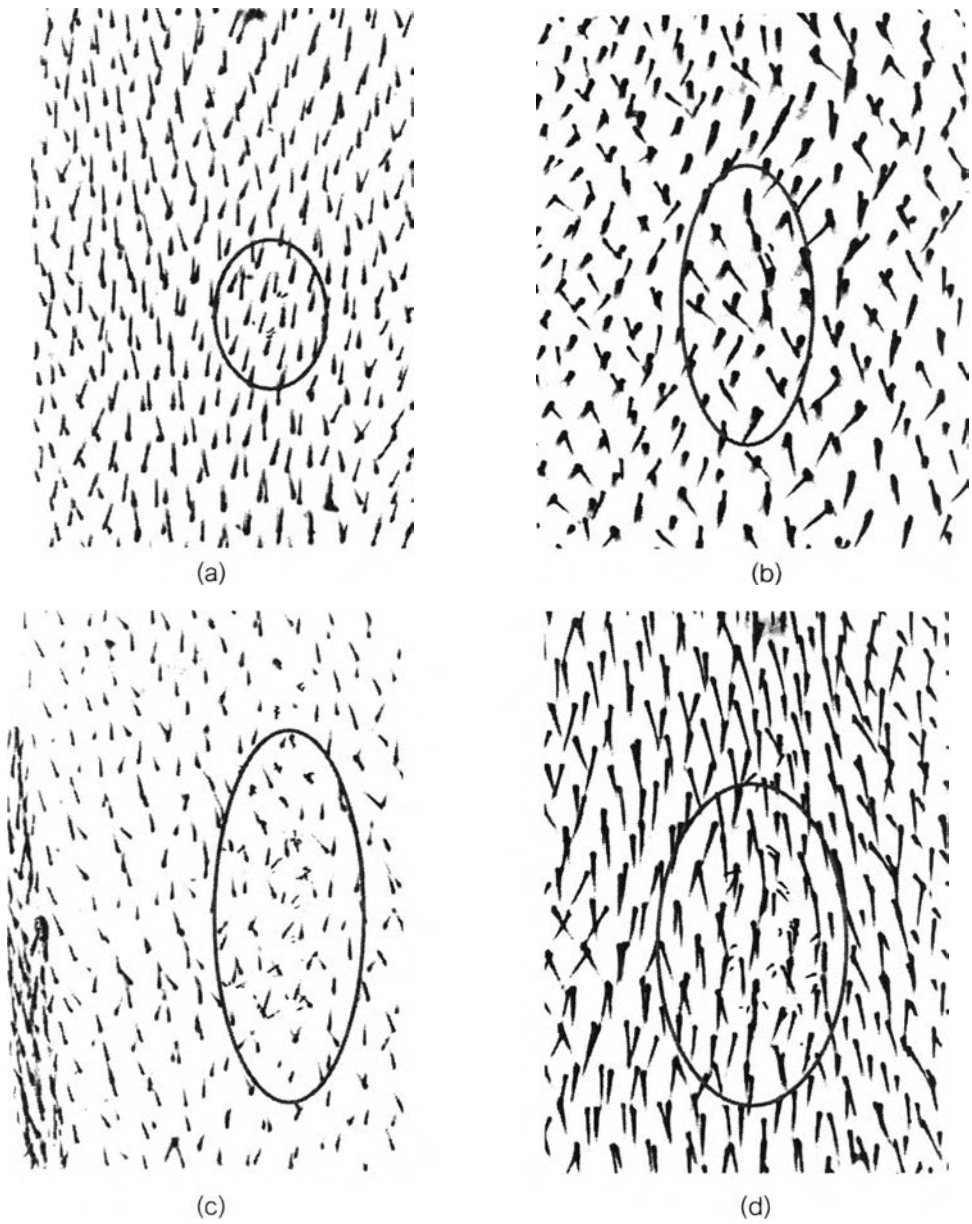


Figure 7 Marker mutations of wing surface to show clone of cuticle secreted by cells homozygous for multiple wing hairs, a) small single spots, b) flare on wing, c) twin spots, d) large single spots

2.6.2 Approach of SMART

Three crosses of flies carrying the marker *mwh* and *flr*^{3} on the left arm of chromosome 3 were generally set up:

1. Standard cross: *flr*^{3} / *TM3*, ser virgin females mated to *mwh* males. This is the reciprocal cross of the standard cross used previously (Moraga and Graf, 1989)

2. High bioactivation (HB) cross: *ORR; flr*^{3}*/TM3*, ser females crossed with *ORR; mwh* males. This is the reciprocal cross of the one described by Frolich and Wurgler (1989). A number of promutagens showed increased genotoxicity when the HB cross was used, compared to standard cross (Frolich and Wurgler, 1989; Frolich and Wurgler, 1990a; Frolich and Wurgler, 1990b). However, the HB cross presents a number of difficulties and disadvantages (Frolich and Wurgler, 1989, Frolich and Wurgler, 1991). These are: (1) The presence of an irregular whorling in the pattern of wing hairs making spot classification difficult, especially for inexperienced scorers, (2) an undesirably high variation in results from repeated experiments, (3) the low egg production of the females used and the delay in development of the larvae of HB cross.

3. Improved HB cross: *ORR, flr*^{3} */TM3*, ser females crossed with *mwh* males (Graf and Van, 1992). The main advantage of the improved HB cross is to combine the high bioactivation capacity with the ease of scoring the wings using the same criteria as for the standard cross. The hybrid larvae of the improved HB cross show P450-dependent activation capacity equal to or even slightly higher than those of the original HB cross. In addition, the IHB cross is more sensitive than the standard cross in evaluating the genotoxicity of promutagens (Graf and Van, 1992).

2.6.3 Application of wing SMART assay

The *Drosophila* wing spot test has been used as well for the study of the modulation of genotoxicity by chemopreventive agents. Negishi *et al.* (1989) for the first time showed that chlorophyll and Cu-chlorphyllin inhibit Trp-P-2-mutagenicity in the wing spot test, on feeding the larvae with the green pigments together with Trp-P-2. Later, with the use of the *Drosophila* wing spot test, protective effects of chlorophyllin against γ-ray irradiation (Zimmering *et al.*, 1990), against chromium mutagenesis (Olvera *et al.*, 1993)

and against various carcinogenic mutagens were shown (Negishi *et al.*, 1994). Various antimutagens such as sodium thiosulphate (Katz, 1989), ascorbic acid (Olvera *et al.*, 1995), instant coffee (Abraham and Graf, 1996), and antioxidant (Karekar, Josh, and Shinde, 2000) have modulating effects on genotoxic agents in the wing somatic cells of *Drosophila*.

2.6.4 Limitation of the test

An important technique problem is the selection of the route of application. Depending on the aim of the experiment and on the chemical properties of the compound under test, the route of application has to be selected with care, in order to assure that a significant exposure to the chemical or to its metabolites is achieved. Occasionally problems were encountered with water-insoluble compounds and the use of DMSO as solvent (Wurgler, and Vogel, 1986). In addition, the problems with the male feeding routine procedure were the low sensitivity of particular chemicals such as aromatic amines and polycyclic hydrocarbons (Valencia and Houtchens, 1980). There are several possibilities to improve the detection capacity of the *Drosophila* system.

a) Larvae feeding: Feeding male larvae instead of adult males seems to develop in to a very attractive alternative. Certain polycyclic hydrocarbons which have been missed with the male feeding procedure can be picked up by larvae feeding. One of the important points seems to be the enormous amount of food which the larvae take up during the day 4 of larvae life. With the food the larvae may also take up large quantities of chemicals even if these are present as small particles as in the case of water insoluble compounds. With promutagens certain differences in the xenobiotics metabolizing systems between larvae and adults may also play an important role (Vogel, 1977; Nigsch, 1978). Larvae feeding may also be the technique of choice if chemicals have to be tested, which are suspected to yield only or predominantly delayed mutations.

b) Enzyme induction: It is possible to improve the xenobiotics metabolizing capacity in transgenic *Drosophila*, after the first demonstration by Magnusson and co-workers (1990), under intensive study in several laboratories.

c) Stock differences: Stock differences with respect to the P-450 composition have been detected (Baars, 1980). For example, with vinyl chloride distinct

strain differences in the enzyme induction and activation capacity were observed (Magnusson and Ramel, 1990). This observation pointed to the possibility that particular suitable stocks may be constructed, which would allow to optimize the detection capacity of *Drosophila* for promutagens.

2.7 Standard Mutagens for Mutagenicity of SMART

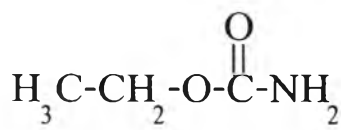
Urethane is generally used as positive standard toxicants in evaluation genotoxicity of the unknown compounds in SMART (Abraham and Graf, 1996). These chemical required metabolic activation to express their mutagenic activity (Frolich and Wurgler, 1990b). It is known as ethyl carbamate. Urethane may 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 anaesthetic for laboratory animals. In 1943, it was discovered that urethane had a carcinogenic effect in animals. It is regarded as an initiator, but also as a co-carcinogen and specifically as a promoter of radiation-induced cancer (Berenblum, 1982). Since 1948 it has been known that urethane is mutagenic in *Drosophila melanogaster*. Today, humans are exposed to urethane in their food and mainly alcoholic beverages.

2.7.1 Metabolic Activation and Detoxification of Urethane

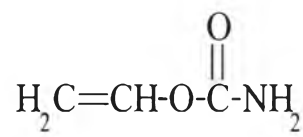
Urethane induced point mutation, gene conversion, intrachromosomal recombination, chromosomal aberrations and sister chromatid exchanges in yeast, plant systems and mammalian cells (Schlatter and Lutz, 1990). Urethane exerts its carcinogenic effect following bioactivation to vinyl carbamate and then to vinyl carbamate epoxide which forms RNA and DNA adducts and initiates tumorigenesis (Dahl, Miler, and Miler, 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, Kim, and Iwasaki, 1991). The schematic structures of urethane and its metabolites show in Figure 8. Urethane is metabolized by two different pathways (Figure 9). The major pathway,

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.

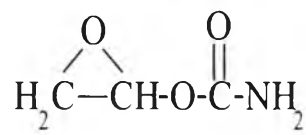
The minor pathway involves the oxidation of urethane via cytochrome P-450 subtype 2E1 (CYP2E1) to 2-hydroxyethyl carbamate, to *N*-hydroxyethyl carbamate and to vinyl carbamate, which is in turn converted by epoxidation to the putative ultimate carcinogen vinyl carbamate epoxide (Miller and Miller, 1983; Guengerich *et al*, 1991; Guengerich and Kim, 1991). 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).



(a)



(b)



(c)

Figure 8 Schematic structures of urethane ant its metabolites (a) Urethane (ethyl carbamate); (b) Vinyl carbamate; (c) Vinyl carbamate epoxide.

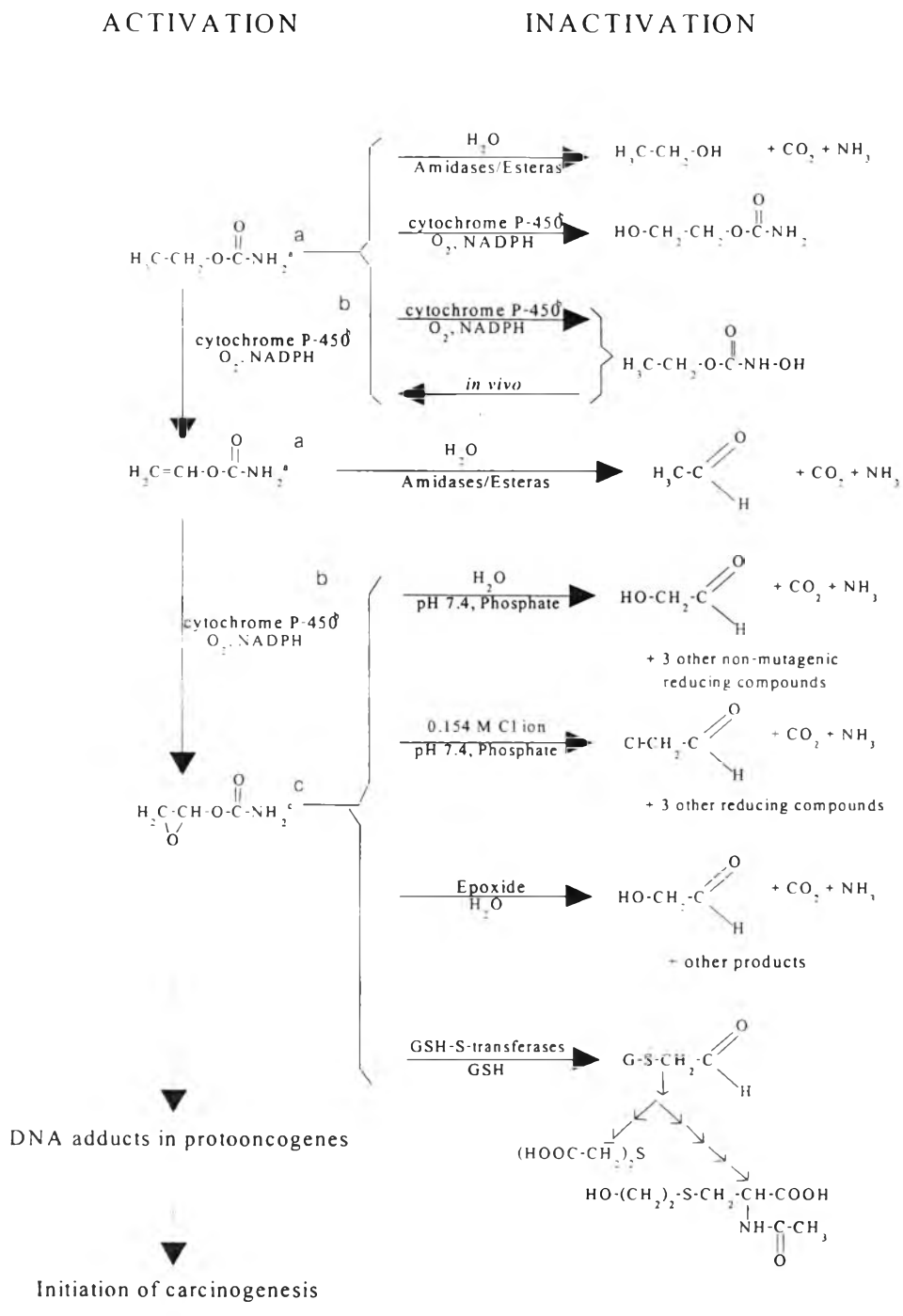


Figure 9 Known and probable activation and inactivation pathways of metabolism of urethane (ethyl carbamate), vinyl carbamate and vinyl carbamate epoxide. (a)Mouse liver microsomes + ethylcarbamate or vinyl carbamate + adenosine 1,N^6-ethenoadenosine.(b) Human liver microsomal cytochrome P450 IIE1.(c) Vinyl carbamate epoxide + adenosine 1,N^6-ethenoadenosine. GSH = glutathione (Park *et al.*, 1993).

2.7.2 Mutagenicity of Urethane

Many studies were published concerning the mutagenicity of urethane in a wide range of organisms (Field and Lang, 1988; Kada and Ishidate, 1980). In tests with eukaryotic cells, positive and negative findings were about equal in frequency. It seems that positive results are obtained only under conditions of appropriate metabolic activation. Urethane is 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 are altered (constitutively increased P-450 enzyme activities) (Frolich and Wurgler, 1990b; Frolich and Wurgler, 1988). The effects are dose-dependent and the modified strain (ORR) is more sensitive to urethane by about one order of magnitude than the standard strain. This further suggests that the P-450 enzyme system is involved in the activation of urethane. Several authors proposed a metabolic pathway which leads to the formation of vinyl carbamate and, after epoxidation, to DNA and RNA adduct (Leithauser *et al.*, 1990; Miller and Miller, 1983) In addition, there is a vast literature on urethane carcinogenicity (Mirvish, 1968; NCI, 1978; NCI, 1979-80) and urethane is a pluripotent carcinogen with respect to tumor induction in different species, organs, and the stages of development of the animals (Zimmerli and Schlatter, 1991).

2.7.3 Alteration Mutagenicity of Urethane

A number of compounds are good inducers of cytochrome P-450 subtype 2E1 (CYP2E1) including ethanol that can increase the metabolism of urethane or decrease it depending upon the condition of exposure (Kurata *et al.*, 1991; Yamamoto *et al.*, 1990). Acute administration of high doses of ethanol may postpone the metabolism of urethane, possibly by blocking metabolizing enzymes, including the group of cytochrome P450 (Waddell, Marlowe, and Pierce, 1987; Yamamoto *et al.*, 1988). Chronic administration of ethanol, in contrast to the acute situation, may lead to induction of metabolizing enzymes systems such as P-450 (Lieber *et al.*, 1987) and thus modulated the carcinogenicity of urethane. Mirvish (1968) reported that degradation of urethane was inhibited up to 90 % by blocking esterase activity, which indicated that ethanol may be formed in near

equimolar amounts to the administered urethane dose. It remains to be shown whether the ethanol thus formed can modulated the further metabolism of urethane. Kurata *et al.* (1991) tested the effects of acetone on metabolism of ethyl carbamate given concurrently in mice. In this study, acetone inhibited the metabolism of ethyl carbamate. Carlson (1994) showed that the cytochrome P-450 inducers (phenobarbital and γ-naphtholflavone) and esterase inhibitor (paraoxon) were without effect (to the conversion of carbonyl-^{14}C urethane to $^{14}CO_2$) whiles the CYP2E1 inhibitor (diethyldithiocarbamate) decreased metabolism to about 3% of control. Ethanol administered acutely inhibited urethane metabolism. Pyridine, showed previously to enhance this metabolism in microsomal preparations, greatly inhibited it *in vivo* (Carlson, 1994).

Kemper, Myer, and Hurst (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, a measure of vinyl carbamate epoxide toxicity, in a concentration-dependent manner at concentration ranging from 1 to 8 mM. This effect was significantly enhanced by addition of rat liver glutathione *S*-transferase. In addition, pretreatment of mice with 1% dietary butylated hydroxyanisole (BHA) caused parallel increases in cytosolic glutathione *S*-transferase activity and cytosolic enhancement of detoxification of vinyl carbamate epoxide by glutathione.

The major conclusions of the study were (1) vinyl carbamate epoxide could be detoxified by spontaneous conjugation with glutathione, (2) conjugation of vinyl carbamate epoxide with glutathione could be catalyzed by glutathione *S*-transferase, (3) pretreatment with BHA protected against binding of active urethane metabolites *in vitro* and *in vivo*, and (4) the protective effect of BHA against urethane metabolite was mediated by increases in glutathione *S*-transferase activity and glutathione concentration. De flora *et al.* (1986) reported that *N*-acetylcysteine (NAC), a precursor of intracellular glutathione, efficiently prevented the induction of lung tumors in Swiss albino mice, when supplemented to the diet both before and after and i.p. injection of the carcinogen urethane. Irrespective of urethane administration, NAC also significantly enhanced glutathione *S*-transferase (GST) activity in liver preparations of the same animals.

Investigation on the change in glutathione *S*-transferase activity in relation to the observed *in vivo* antigenotoxicity of fresh vegetables, spices, tea and coffee was done by Abraham, Singh, and Kesavan (1998). This experiment showed that treatment with urethane alone resulted in inhibition of glutathione *S*-transferase activity. Co-adminstration of URE with extracts of vegetables, coffee and spices resulted in dosed-related attenuation of the inhibitory effect of URE on GST activity. However, tea had no effect on inhibition of GST activity by URE. Hence, and association between antigenotoxicity and GST activity could not be established.

A dose-dependent increase in the genotoxic activity of urethane was observed in SMART (Frolich and Wurgler, 1990b). The frequency of induction of mutations in the modification strain with increased P-450 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 (Frolich and Wurgler, 1990a). This might result from the constitutive expression of the enzymes required for the transformation of urethane into ultimate genotoxic metabolites.