CHAPTER V

DISCUSSION

Gastric cancer prevention was the most desirable route for cancer control, while chemoprevention was a promising alternative to reduction of the inevitable human exposure to dietary carcinogen. Particularly nitrite intake and gastric mortality in various countries has been documented. Much of the evidence for the role of diet in cancer has been interpreted as evidence for the presence of carcinogenic factors in the diet (Lynnette, 1999). For example, the reaction between nitrite and dietary amines and amides under the acidic conditions of the stomach could lead to the formation of nitrosated products. It was possible to induce gastric cancer in human (Mirvish, 1983). On the other hand, it is well known that ingredients in diet including edible plants, fruits and seeds may exert anticarcinogenic and antimutagenic activities (Hartman and Shankel, 1990; Hayatsu *et al.*, 1988). Therefore, the effects of the selected five vegetables on the mutagenicity of nitrite treated 1-AP and nitrite treated beef extract, in the absence of metabolic activation, were examined using the Ames test.

5.1 Mutagenicity of Nitrite Treated 1-Aminopyrene and Nitrite treated Beef Extract

In Ames test, 1-AP treated with excess of sodium nitrite in acid solution (pH 3.0) for 4 hr was used as a positive mutagen and as an example of mutagen occurring during the gastric digestion. The products of nitrite treated 1-AP, 1-nitropyrne and unidentified nitro-introduced compounds which revealed their direct mutagenicity towards *S. typhimurium* TA98 and TA100 (Kato *et al.*, 1991: Kangsadalampai, Butryee, Manoonphol, 1996). Since the products of the model did not require metabolic activation before expressing their mutagenicity, it allowed us to determine the counteracting activity of the sample on the mutagen that may cause mutation to the gastric cells. The

above model was used to determine whether there were some antimutagens from various substances against the mutagen in the gastric like pH solution.

The product of nitrite treated beef extract was selected because it showed the mutagenicity towards *S. typhimurium* both TA98 and TA100 strains in the absence of metabolic activation. The mutagenic products formed during the reaction of beef extract and sodium nitrite were proved to be the nitro-heterocyclic amine compounds (Tsuda *et* al., 1985; Munzner, 1986). Cooking of protein rich foods may induced the formation of mutagens and carcinogens (Stavric, 1994). The mutagenic potential of beef extract was prepared from boiling meat for 8 hr and subsequently being interacted with nitrite was found on *S. thyphimurium* TA98 and TA100 in this study. It was demonstrated that extensive length of cooking time at habitual household cooking condition such as boiling could produce indirect mutagens that caused mutation towards *S.typhimurium* TA98 in the presence of activating system (Commoner *et al*, 1978). The finding of direct mutagenic activity in beef extract studied by Commoner *et al* suggested that sample contained some convertible precursors. The mutagenic products from the reaction between nitrite and beef extract were speculated to be direct mutagenic nitro-compounds (De Meester, 1989). These substances might be derived from the reaction between IQ-type heterocyclic amines which occurred during boiling meat and nitrite as proposed by previous studies (Sasagawa *et al*, 1988; Lin *et al*, 1992; Peerawong and Kangsadalampai, 1998). Sasagawa *et al*.(1988) reported that treatment of IQ type heterocyclic amines with nitrite (50 mM) resulted in conversion of amino groups to nitro groups. The nitro derivatives of imidazoquinoline and imidazoquinoxaline showed almost the same mutagenicity in the absence and presence of metaboliuc activation as parent amino derivatives.

5.2 Mutagenicity of Chloroform and Ethanol Extract from the Vegetables

Generally, all of the chloroform and ethanol extracts from vegetables in this study, except chloroform extract of Pak Ka Yang (0.190 mg/plate) on TA98, were not mutagenic on both *S. typhimurium* TA98 and TA100 in the absence of metabolic activating system. This information, therefore, supported that Thai indigenous

vegetables are safe. Pak Ka Yang (0.190 mg/plate) was mutagenic hence, it might contain some mutagens. On the other hand, both nitrite treated chloroform and ethanol extracts of all the vegetables showed mutagenicity as in the experiments of Intiyot *et al* (2002), Chiampanichayakul *et al* (2001) and Vachalkova *et al* (2000). That should be the precaution to the health concerning consumers in simultaneously consuming such vegetables along with nitrite containing food items.

5.3 Effect of Chloroform and Ethanol Extracts from the Vegetables on Mutagenicity of Nitrite Treated 1-Aminopyrene.

The present study is the attempt to elucidate the protective effect of extracts of the vegetables on the tester strains against mutation induced by nitrite treated 1-AP and nitrite treated beef extract. The chloroform and ethanol extracts from all vegetables, except ethanol extract from Pak Gud, showed the antimutagenic effect on the mutagenicity of nitrite treated 1-AP. The result indicated that the direct acting mutagens formed during interaction between nitrite and 1-AP could be suppressed by some components in the extracts. Such compounds extracted were from vegetables were flavonoids (Nakaoki and Morita 1960; Shigematsu, Kouno and Kawano, 1982; Gabriele, Hiller and Franke, 1981), glycosides (Della Greca *et al*, 1993), triterpenoids (Della Greca *et al*, 1994; Kwon, Zee and Lee, 1998), and chlorophyll (Chansakaow, Ruangrungsi and Ishikawa, 1996). Therefore, the possible mechanisms of antimutagenicity of vegetable extracts might be explained as the following.

Firstly, some flavonoids in the vegetable extracts might inhibit enzyme in the bacterial cell, nitroreductase and/or O-acetyltransferase in *Salmonella* cell as the activating enzymes of many direct mutagens. It was speculated to be the target on study of inhibition by Edenharder and Tang (1997). Flavonoids were common compound in plant. There were researches found flavonoids compound in Pak Chee Lao (Niyazawa and Kameoka, 1974) Pak Ka Yang (Krishnan, Nair and Ramachandran, 1999) and Pak Pai (Srivastava *et al.*, 1981). Lee and Lin (1992) found that flavonoids could inhibit nitroreductase. In the bacterial tester strains (TA98 and TA100) flavonoids inhibited the activity of mutagens identified in foods whether it was direct acting [e.g. 2-

aminofluorene] or it required metabolic activation [2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) and 2-amino-3-methylimi-dazo[4,5-*f*]quinoline (IQ) and 3-amino-1,4-dimethyl-5H-pyrido[4,3-*b*]indole (Trp-P-1) (Edenharder, Rauscher and Platt, 1997; Calomme, 1996; Weisburger, Dolan and Pittman, 1998 and Miyazawa *et al.*, 1999).

Secondly, it was proposed that vegetable extracts may modify the permeability of mutagen across bacterial membranes. Edenhader and Tang (1997) reported that 1-nitropyrene was in general more effectively antagonized by potent antimutagenic flavonoids. They suggested that different mechanism of antimutagenesis were at work. Antimutagenic flavonoids might modulate the mutagenic response of the nitroarenes, which have 1-nitropyrene as a member. However, this suggested a hypothesis still required to be proved.

Thirdly, complex formation between vegetable extracts and a mutagen might limit the bioavailability of mutagens to the tester bacteria. Stavric (1994) suggested that fiber, polyphenolic compounds, chlorophyll and calcium could form complexes with mutagen in the intestinal tract. These compounds were generally found in vegetables. Birosova, Mikulasova and Vaverkova (2005) studied the antimutagenic effect of phenolic acids on 3-(5-nitro-2-furyl)acrylic (5NFAA) found the antimutagenicity of caffeic and ferulic acids extracted from plant species of genera *Echinacea*(L) on 5NFAA and cichoric acid from that plant increased the mutagenic effect on 5NFAA. Niyazawa and Kameoka (1974) and Krishnan, Nair and Ramachandran (1999) found that Pak Chee Lao and Pak Ka Yang contains polyphenol compounds such as limonene, thymol, myrcene etc. Limonene from *Urtica sp* and rosehip inhibited mutagenicity of sodium azide (Karakaya and Kavas, 1999). It was not found the mutagenicity on *S. typhimurium* TA100. It should be the formation of a complex between phenolic compounds and mutagen, which could limit the bioavailability of mutagen. This may describe the mechanism how extract of vegetables in the present experiment inhibited the mutagenesis of nitrite treated 1-AP.

5.4 Effect of Chloroform and Ethanol Extracts from the Vegetables on Mutagenicity of Nitrite Treated Beef Extract.

The present result indicated that the direct acting mutagens formed during interaction between nitrite and beef extract could be suppressed or enhanced by the components in the vegetable extracts. These results suggest that some mutagens and carcinogens may be produced in the human stomach. In this experiment, vegetable extracts showed inhibiting effect on the mutagenicity induced by nitrite treated beef extract. There were many research found that vegetables contain compounds that have the antimutagenicity (Edenharder and Tang, 1997; De Mejia, Quintanar-Hernandez, and Loarca-Pina, 1998). It suggested that the antimutagenicity of vegetable extracts may due to compounds that could counteract the direct acting mutagen. There were many natural antimutagens such as chlorophyll and its derivatives (Edenharder, Leopold and Kries, 1995), Polyphenols (Hayatsu, Arimoto, and Neigishi, 1988). Nakahara and Trakoontivakorn (1998) were reported that Pak Pai suppressed the mutagenicity of Trp-P-1, an indirect mutagen required metabolic activation. The antimutagenic agent(s) was considered to be present in the extraction of the vegetables. In this study, we found that chloroform and ethanol extracts of Pak Ka Yang and Pak Pai could inhibit mutagenicity of nitrite treated beef extract, a direct mutagen, on the absence activation. The antimutagenic effect of these vegetables might due to inhibition effect of some compounds in Pak Ka Yang and Pak Pai on bacterial enzymes. The mechanisms or compounds of vegetables that showed antimutagenicity on direct mutagen might be more investigation.

On the other hand, we found that the chloroform extracts of Pak Chee Lao, Pak Gud and Pak Krad Hua Wan and the ethanol extracts of all vegetables enhanced mutagenic activity of nitrite treated beef extract. Ferguson (2001) reported that many of the chemicals described as antimutagens may also act as co-mutagen. Some constituents of the vegetable extracts may increase the mutagenic efficiency with an unknown mechanism that was not specific to the mutagen. Tang and Edenharder (1997) reported that the juices from several fruits exerted weak to moderate activity with

respect to mutagenicity induced by nitroarenes in *S. typhimurium* TA98. It was found that when some vegetable extracts were incorporated into the pre-incubation tube composed of nitrite treated beef extract and the tester strain, they increased the number of revertants of *S. typhimurium* strain TA98 higher than that of the positive control tube.

The inductions of vegetable extracts on enzyme, namely, nitroreductase and O-acetyltransferase in *S. typhimurium* of Ames test that are believed to play an important role in mutagenesis of positive standard should be investigated. In addition, the antimutagenicity of vegetable extracts should be performed on other testing systems in order to warrant the effect of the vegetables found in the present experiment. Finally, the differences between bacterial cells (*in vitro*) and mammalian tissues (*in vivo*) might be limit in interpretation of such benefit of vegetables; therefore, examinations for their antimutagenic activity in animal model are recommended.

Beef is the most popular meat used in Northeastern style cooking. Concerning public food safety, beef is noted for its mutagenicity, generated when treated with nitrite under strongly acidic solution in relation to the high incidence of cancer in America (WCRF/AICR, 1997). The present results indicated that beef extract was mutagenic after treating with nitrite. Therefore, these might be the possible risk of gastric cancer in consumers that consume both nitrite and meat product. Surprisingly, people in northeastern area of Thailand have low incidence of gastric cancer (NIC, 2000). It might due to their consuming behavior. The regional foods, Kang Aom(แกงอ่อม), Tom Sab(ต้มแซ่บ), Lab(ลาบ), Spicy Salad(ยำ), contained meat and many kinds of vegetables such as Pak Chee Lao, Pak Ka Yang, Pak Pai, Pak Gud, and Pak Krad Hua Wan. In this study revealed that chloroform and ethanol extracts of Pak Ka Yang and Pak Pai inhibited mutagenicity on nitrite treated beef extract. On the other hand, some vegetable extracts showed enhancing mutagenicity on nitrite treated beef extract. It suggested that these vegetables may have some useful and/or hazardous compounds for consumers. In conclusion, the one way to prevent gastric cancer is to consume more and various vegetables.