CHAPTER V

DISCUSSION

5.1 Mutagenicity of Beef Concentrate

The beef concentrate, prepared from prolonged boiling for 6 hours, treated with nitrite at pH 3 and 37°C for 4 hours showed its mutagenicity towards *S. typhimurium* TA98 and TA100 in the absence of metabolic activation (Tables 3 and 4). From this condition, the direct mutagen compounds that could induce frameshift mutation and base-pair substitution were formed. Several studies showed that the direct mutagen compounds formed during the reaction of the beef extract and sodium nitrite in the gastric-like condition were heterocyclic amine compounds (Tsuda *et al.*, 1985; Sugimura *et al.*, 1977; Münzner, 1986). 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 (Lin *et al.*, 1992; Sasagawa *et al.*, 1988). The increase of revertants per plate of beef concentrate on *S. typhimurium* TA100 may be due to free histidine contained in beef concentrate.

5.2 Mutagenicity of Extracts from Edible Mushrooms

Results of this study demonstrated that these edible mushroom extracts namely, button, shiitake, oyster, and abalone extracts did not contain any compound(s) directly mutagnic towards *S. typhimurium* either strain TA98 or TA100. Treated with nitrite under acidic condition, mushroom extracts showed mutagenicity on both strains of *S. typhimurium* TA98 and TA100 (Tables 5 and 6). It was suggested that they might contain some compounds that could further react with nitrite to produce direct mutagenic products causing frameshift and base pair substitute mutations. The direct mutagenic products may be nitro-compounds that derived from the reaction between edible mushroom extracts and nitrite. In

addition, this investigation presented that button mushroom extract had the highest mutagenic activity compared with those of the other three mushroom extracts on *S. typhimurium* TA100. It may be the reason that there was high concentration of agaritine in the button mushroom [up to 300 mg/kg fresh weight (Sharman *et al.*, 1990)]. Agaritine is suspected of having a genotoxic potential because of its structural similarity to known carcinogenic hydrazines (Toth, 1980; Toth and Nagel, 1981). It gave a weak mutagenic response only *S. typhimurium* strain TA2637, whereas *A. bisporus* extract gave response with strains TA98, TA2637 and TA100. The most sensitive was found on TA100. From this study, button mushroom extract also gave the most sensitive on TA100 after nitrite treatment. It is proposed that agaritine might interact with nitrite and posed higher mutagenicity; further experiment on the mutagenicity of nitrite treated agaritine should be done.

5.3 Mutagenicity of Concentrate from Beef Boiled with Various Amounts of Some Edible Mushrooms

The finding of this study demonstrated that none of the concentrate of beef boiled with edible mushrooms had direct mutagens that caused frameshift mutation or base pair substitution mutation (Tables 7 and 8). Indirect mutagens in meat extracts formed during normal cooking were identified as heterocyclic amine (Starvic, 1994). Heterocyclic amines formed at temperatures below 300^{0}C are often referred to as thermic mutagens or IQ-type (Sugimura *et al*, 1989; Skog, 1993). They must undergo metabolic activation to become reaction species possessing mutagenic and/or carcinogenic capacities (Starvic, 1994). This may be explain why all samples were not mutagenic on both TA98 and TA100 in the absence of metabolic activation in gastric like condition in this experiment. After nitrite treatment, almost all samples were mutagenic on both *S. typhimurium* TA98 and TA100. Because the mutagenic products from the reaction between nitrite and concentrate from beef boiled with edible mushrooms were speculated

to contain 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).

The color of concentrate from beef boiled with edible mushrooms was deep brown. This color was supposed to be non-enzymatic reaction or Millard reaction products occurred during prolong heat processing (Kaanane and Labuza, 1989). Yen and Lee (1986) reported that nitrosation of products from Maillard reaction showed strong mutagenicity to all tester strains (TA97, TA98, TA100, TA102 and TA104) both in the presence and absence of the metabolic system.

Edible mushrooms are a potential source of dietary fibers: fungal cell walls contain chitin, other hemicelluloses, mannans and beta glucans (Cheung, 1998). Manzi *et al.* (2001) reported that button mushroom (*Agaricus bisporus*) and oyster mushroom (*Pleurotus ostreatus*) presented dietary fiber in amounts of 1.98 and 4.10 g/100g of edible weight, respectively. In addition, Yang *et al.* (2001) found that abalone mushroom (*Pleurotus cystidiosus*) and oyster mushroom contained crude fiber amounts of 8.74 and 5.33 % based on dry weight respectively. Several studies have reported that dietary fiber from plant cell walls could adsorp heterocyclic amines (HAs) such as IQ, MeIQ, MeIQx, PhIP, Glu-P-1, Glu-P-2, Trp-P1 and Trp-P-2 (Ferguson *et al.*, 2003; Harris *et al.*, 1996; Takeuchi *et al.*, 1988). Moreover, these mushrooms contain a variety of secondary metabolites, including various phenolic compounds, which have been shown to act as the excellent antioxidants (Ishikawa, Morimoto and Hamasaki, 1984; Mau, Lin and Song, 2002). Positive correlations were found between total phenolic content in the mushrooms and their antioxidant activities (Cheung *et al.*, 2003; Dubost, Ou and Beelman, 2007; Yang *et al.*, 2002). Antioxidants could reduce the formation of HAs which may act as free radical scavengers (Britt *et al.*, 1998) and thus interfere with the HA-forming pathways.

From this study, it was found that the addition of edible mushrooms during extend period cooking could not reduce the formation of direct mutagen precursor that caused frameshift or base pair substitute mutation (Figures 9-12 and Table 9). This may be due to dried mushrooms used in this study were not homogeneous and substances contained in different part of mushroom did not the same substances.

5.4 Effect of Extracts from Edible Mushrooms on the Mutagenicity of Beef Concentrate with and without Nitrite Treatment

The finding of this study demonstrated that none of the mixtures of mushroom extract and beef concentrate had direct mutagens that caused frameshift or base pair substitute mutations. After nitrite treatment, all mixtures had mutagenic effect on both tester strains (Tables 10 and 11). Then, the mutagenic products from the reaction between nitrite and mixtures of beef concentrate and mushroom extracts were speculated to contain direct mutagenic nitro-compounds (De Meester, 1989). However, it was found that the actual MIs of nitrosated mixtures of beef concentrate and mushroom extract were lower than the summation of MIs of nitrosated beef concentrate and each mushroom extract separately (expected MI) (Tables 12 and 13). It is proposed that the reduction of the mutagenicity of the nitrosated mixture of beef concentrate and mushroom extracts may be due to the competition between the beef concentrate and mushroom extracts in interacting with nitrite in the reaction mixture. In addition, these mushrooms contain various phenolic compounds, which have been shown to act as the excellent antioxidants (Cheung *et al.*, 2003; Dubost *et al.*, 2007; Yang *et al.*, 2002). Dubost *et al.* (2007) reported that the predominate phenolics identified in the button mushroom included tyrosine, catechol, and the phenolic acids, *p*-hydroxy-benzoic acid, *p*-coumaric acid and vanillic acid, based on the ability of phenol oxidase to oxidize various phenolic compounds. The oyster mushroom contained

the phenolic compounds, syringaldehyde, guaiacol and catechol with no detection of tyrosine. Some simple phenols and phenolic compounds can reduce the activity of nitrite in the formation of mutagenic species depending on their structure and reaction conditions. Under acidic conditions, phenolics usually react with nitrite more rapidly than most amino compounds. 1,2- and 1,4-dihydroxyphenols, such as catechol, hydroquinone, 1,2,3-trihydroxyphenols (e.g. pyrogallol, gallic acid), *para*-substituted phenolics (e.g. vanilline) and many naturally occurring polyphenols (e.g. phenolic, cinnamic and chlorogenic acids, tannins), are oxidized to quinoid derivatives and inhibit nitro-compounds formation from the reaction between IQ-type heterocyclic amines contained in beef concentrate and nitrite (Bartsch *et al.*, 1988; Lin *et al.*, 1992; Sasagawa *et al.*, 1988). However, the amount of nitrite was excess and should be sufficient to react with polyphenol; therefore, this mechanism was probably not the cause in reducing the mutagenicity of the mushroom extract and beef concentrate mixture.

Another possible mechanism was that the mutagenic products of the mushroom extracts and beef concentrate after nitrite treatment were competitive in interacting with the tester strain DNA. However, the study on button and oyster mushroom extracts through TA98 at the higher doses presented different results in term of synergistic effect; the actual MIs of these mushroom extracts were higher than the expected ones. It probably implied that such newly formed mutagens had different characteristic of interaction depended on dose of the mushroom extract i.e. low dose gave antagonistic activity but high dose gave synergistic activity. Further experiment to prove this mechanism is required.