CHAPTER V

DISCUSSIONS

5.1 Antioxidant Assays

Overall of the study indicated that Hom Nil rice and black glutinous rice and their products are good sources of antioxidant. Several studies revealed that there were antioxidant and phenolic compounds in black glutinous rice (Chanphrom, 2007; Htwe, 2009; Tananuwong and Tewaruth, 2010; Tewaruth, 2007) and Hom Nil rice (Kukam-oo *et al.*, 2008; Nakornriab *et al.*, 2008). The reason that black glutinous rice extracts had higher antioxidant activity than that of Hom Nil rice extracts was possibly due to the darker color of the black glutinous rice extract. It was proposed that the difference in color might be due to the amount of anthocyanins (สายสนม ประดิษฐ์ดวง, 2551). The results from this study were similar to that of Choi *et al.* (2007); they also reported that black rice had 87% radical scavenging activity contained 313 ± 31.5 mg gallic acid equivalents per 100 g polyphenolics in the methanolic extract and had anthocyanins as the major extractable polyphenols.

The result that cooked rice had less antioxidant activity than that of raw rice might indicate the effect of cooking on rice component. Chanphrom (2007) reported that the antioxidant activities, total polyphenolic compounds and anthocyanidins in purple rice declined 46.4% during cooking. This suggested that some of the polyphenolic such as anthocyanin was more sensitive to heat from food processing (Markakis, 1982; Larrauri *et al.*, 1997).

The reason why fermented product of any rice had the lowest antioxidant and total phenolic compounds compared with the raw and cooked ones (based on 8 mg/ml of each rice extract) might be due to the fact that during fermentation the starch was digested to reducing sugar (Ülgen *et al.*, 2002; Horn *et al.*, 1992). This reducing sugar could be extracted and increased the percentage yield while the starch of raw and cooked rice was not extracted by ethanol in the process of determination. This resulted in lower yield of the extract of raw and cooked rice that made the concentration of antioxidant and phenolic compounds increased, compared to the same amount of the extract.

Considering based on dry weight basis of the starting materials, fermented samples namely, FH and FB possessed the highest on both antioxidant activity and the phenolic contents. Đorđević *et al.* (2009) indicated that fermentation could enhance the levels of antioxidant activity and also improve the bioactive potential of the rice as suggested by Lee and Chou (2006) who reported that fermentation increased the content of aglycone e.g. the bioactive isoflavone of the final product. Thus, fermented Hom Nil rice and black glutinous rice might be useful to use as antioxidant in food industry.

5.2 Mutagenicity of Rice Extracts

The results that black glutinous rice and Hom Nil rice extracts expressed their direct mutagenicity only after being treated with sodium nitrite in Ames test suggested that the samples contained some precursors that could react with sodium nitrite under acid condition. The products caused frame-shift mutation and base-pair substitution in *Salmonella typhimurium* TA98 and TA100 respectively. This investigation was nearly the same that reported by Hayatsu and Hayatsu (1989) who treated an aqueous homogenate of boiled Japanese rice with nitrous acid at pH 3 and found that mutagens were formed by isolating the mutagenic fractions through blue-rayon adsorption, a method used to extract polycyclic compounds, and subsequent high-performance liquid chromatography. The mutagens were also active in both *S. typhimurium* strains TA 98 and TA 100 without metabolic activation which was the same phenomenon found in the present report. In addition, they also reported that the metabolic activation system of Ames test greatly decreased the mutagenicity of the nitrite-treated sample. The mutagen precursors of the rice extracts may be indole derivatives that were known to form mutagens after nitrite treatment (Ochiai *et al.*, 1986, Ajarayasiri and Chaiseri, 2008, Dashwood, 2002, Yang *et al.*, 2008). Tryptophan, indole derivatives that possesses the aroma in the rice, is one of amino acids presenting in rice which can form mutagens when being treated with sodium nitrite in acid condition (Gatehouse and Wedd, 1983, Ochiai *et al.*, 1986, Sasagawa and Matsushima, 1989). Although the mutagenicity of rice extracts was detected after nitrite treatment, it does not suggest the risk of rice consumption of the Thai people, since most of Thai dishes served with rice do not contain nitrite as western fermented meat products which generally function as a nitrosating agent.

The extracts from fermented rice had negative or low mutagenic activity compared with that of the cooked one. It was hypothesized that some mutagen precursors might be converted during fermentation to other compounds that could not react with nitrous acid in the incubation mixture. Thus, fermented rice extracts might possess less concentration of formed mutagen per plate compared to that of the cooked one. The other hypothesis suggested that some compounds might be capable of interfering with the nitration of rice extract were formed during fermentation. Therefore, it is warrant to investigate the existence of the mutagen precursor in the fermented rice extract or the formation of nitrating inhibitor in order to elucidate the mechanism of inhibition.

5.3 Antimutagenicity of the Rice Extracts on Standard Mutagens

The results in this study expressed that chicken extract, 1-aminopyrene, and rice extracts had direct mutagens when being treated with sodium nitrite on both tester strains. Then, the mutagenic products from the reaction between sodium nitrite and mixtures of chicken extract or 1-aminopyrene and rice extract were expected to contain direct mutagens as nitro-compounds (de Meester, 1989). In this study, the mutagenicity of sodium nitrite-treated chicken or sodium nitrite-treated 1-aminopyrene was reduced by adding the rice extracts after 4 hours reaction on *S. typhimurium* TA98 and TA100. However, the antimutagenicity against sodium nitrite-treated 1-aminopyrene (1-nitropyrene and unidentified nitro-introduced compounds) was pronounced stronger than that against sodium nitrite-treated chicken extract (possibly nitro-heterocyclic amine compounds). This might be due to substance specific.

The possible mechanisms that the rice extracts could inhibit mutagenicity of both standard mutagens might be the scavenging of the toxic compounds or/and enzyme inhibition. The rice extracts might consist of some components which could form complexes with the mutagens; therefore, they limited the bioavailability of the mutagens to the bacteria DNA. Nitro-polycyclic aromatic hydrocarbons and nitro-heterocyclic amine compounds required bacterial intracellular metabolic conversion to express their mutagenicity toward bacterial DNA (Einistö *et al.*, 1991; Kappers *et al.*, 2000). Thus, the flavonoids in rice extracts might be the potent antimutagenic compounds against 1-nitropyrene (possibly one of the products occurred during nitrite treatment of 1-aminopyrene) (Edenharder and Tang, 1997) and heterocyclic amine

mutagens (possibly the products occurred during nitrite treatment of chicken extract) (Edenharder *et al.*, 1993; Edenharder *et al.*, 1997) by inhibited bacterial nitropyrene reductase (Kuo *et al.*, 1992) or *O*-acetyltransferase (Edenharder and Tang, 1997).

Anthocyanins, the flavonoid presents in Hom Nil rice or black glutinous rice (สายสนม ประดิษฐ์ดวง, 2551), possessed biological properties such as antimutagenicity in bacterial model. Deguchi *et al.* (2000a) and Deguchi *et al.* (2000) found that a novel purple pigment called hordeumin, a type of anthocyanin-tannin pigment, produced from barley bran-fermented broth, has antimutagenicity effect on mutation of some HAs, benzo[a]pyrene and dimethyl sulfoxide extracts from grilled beef, according to the Ames method using *S. typhimurium* TA98. Yoshimoto *et al.* (2001) also found that anthocyanins which have been purified from the purple sweet potato could inhibit the mutagenicity of heterocyclic amine on *S. typhimurium* TA98. Thus, the extracts of plants containing large amounts of anthocyanins might act as inhibitors of mutagenesis. Anthocyanins might change some bacteria properties and/or scavenge mutagen (Kaneda *et al.*, 2006; Pedreschi and Cisneros-Zevallos, 2006), which could form complexes with the mutagen; therefore, they limited the bioavailability of the mutagen to the bacterial DNA.

In addition, the antimutagenicity of black glutinous rice extracts was higher than that of Hom Nil rice extracts which was concordant with the antioxidant activity and total phenolic contents. Hung *et al.* (2007) studied the antimutagenicity of fermented black soybean extracts against some HAs in *S. typhimurium* TA98 and TA100 and indicated that anthocyanin and phenolic compounds of common black bean (*Phaseolus vulgaris*) were also found to exert antimutagenic effect. The relatively high antimutagenicity activities noted in the fermented black soybean extracts is generally associated with the higher contents of total phenolics, anthocyanins and aglycones in fermented black soybean. Wang *et al.* (2010) also found that the antimutagenicity of the koji extract was associated with its antioxidant; anthocyanin content.

The results from this study suggested that consuming Hom Nil rice or black glutinous rice could counteract the direct mutagen formed from the reaction between sodium nitrite and some compounds existed in cooked meat in stomach.

5.4 Anti-Mutagen Formation Effect of Rice Extracts

An attempt to elucidate the interaction between rice extract and either chicken extract or 1-aminopyrene during nitrite treatment was done. In chicken extract model, fermented rice extracts exhibited inhibitory effect on both *S. typhimurium* strains. The fermented rice extracts might possess some precursors which could inhibit nitration or/and fermentation might increase the content of some active compound aglycone (Đorđević *et al.*, 2009; Lee and Chou, 2006). On the other hand, the extracts from CH could inhibit mutagenicity of nitrite-treated 1-aminopyrene on *S. typhimurium* TA98. The inhibitory effect of CH on mutagenicity of nitrite-treated 1-aminopyrene might be due to some components formed after cooking process that presented in Hom Nil rice which should not be found in black glutinous rice. Therefore, it is warrant to investigate the existence of these components in order to elucidate the mechanism of inhibition. Cooking process could result in higher polyphenol concentration (Turkmen *et al.*, 2005; Faller and Fialho, 2009) and might increase some antimutagenic compounds.

In addition, the observed MIs of nitrite-treated chicken extract in the presence of rice extracts in this study were lower than the expected MI which indicated that the rice extracts possessed the antagonistic characteristic on this model. This surprisingly unexpected effect might be due to the fact that each rice extract could interact with nitrite but expressed lower mutagenicity toward both *S. typhimurium* strains.

Hom Nil rice and black glutinous rice extracts contained high polyphenols and high antioxidant activity that might scavenge or interact with some products occurred during nitrosation of standard mutagen. The nitrating reaction can be inhibited in food system by compounds such as complex mixtures extracted from fruits and vegetables, phenolic compounds, and also phytochemicals (Biaudet *et al.*, 1996; Bartsch *et al.*, 1988). Some antioxidants (e.g. flavonoids) and some amino acids (e.g. L-tryptophan) were reported to limit heterocyclic amine formation under various conditions (Weisburger and Jones, 1990; Yen and Chau, 1993; Yen and Hsieh, 1994; Yen and Lii, 1992; Yen *et al.*, 1992; Oguri *et al.*, 1998). Polyphenols could inhibit the formation of nitroso-proline during *in vivo* gastric nitrosation (Mirvish, 1995), and some compounds with antioxidant properties could inhibit the formation of *N*-nitrosamine (You *et al.*, 1996; Serafini *et al.*, 2002), depending on the reaction conditions and their structures. They can act as nitrite scavengers, which compete with the amine or amide for available nitrosating agents (Biaudet *et al.*, 1996).

The other possible mechanism of this antagonistic effect against nitrite-treated chicken extract might be due to the competition of the mutagens as described by Pengket (2005). Since the nitro-compound of chicken extract in this study required bacterial intracellular metabolic conversion to express their mutagenicity toward bacterial DNA, the addition of rice extracts, which could react with sodium nitrite and also converted to nitro-compound, might compete with the mutagens during the activation in bacterial cells with higher affinity to bacterial enzymes but lower efficacy in DNA binding. Ferguson *et al.* (2004) suggested mechanisms by which dietary antimutagens could protect against mutation that the phenols can inhibit nitrosation and the polyphenols, including anthocyanins, possessed blocking or competition with mutagens.

However, in 1-aminopyrene model, nearly all rice extracts but fermented rice extracts exhibited inhibitory effect on *S. typhimurium* TA100 which might be the possible mechanism described above. On the other hand, nearly all rice extracts but the extracts from CH exhibited enhancing mutagenicity of nitrite-treated 1-aminopyrene on *S. typhimurium* TA98.

The difference between antagonistic effect in nitrite-treated chicken extract model and enhancing effect in nitrite-treated 1-aminopyrene model might be due to the structure of mutagen which could interact with some precursors in the rice extracts and interact with bacterial target site. The exact mechanism(s) of each effect in both models was unknown and required further exploration such as identification on the mutagenic precursors in each mutagen after being converted to be direct-mutagens by nitrite and the interaction with components of rice extracts.

The important flavonoids, anthocyanins, in Hom Nil rice and black glutinous rice might give health benefit to consumers. Hagiwara *et al.* (2001) suggested that anthocyanin might prevent colorectal carcinogenesis induced by some heterocyclic amines. Furthermore, polyphenolic compounds also act as a radical scavengers and chain-breaking antioxidants (Bors *et al.*, 1990) and can quench or prevent the formation of reactive oxygen species (Yang *et al.*, 2001). In various culture systems, anthocyanins have shown the antitoxic and anti-carcinogenic effects such as stimulating the expression of phase II detoxification enzymes, directly scavenging reactive oxygen species (ROS), reducing the formation of oxidative adducts in DNA, inhibiting mutagenesis by environmental toxins and carcinogens, reducing cellular proliferation by modulating signal transduction pathways induction of cell apoptosis,

anti-inflammatory effects, anti-angiogenesis, anti-invasiveness and induction of differentiation (Wang and Stoner, 2008). In addition, anthocyanins are able to reduce the oxidative stress from NO, which increases the protective effects against cardiovascular and chronic inflammatory diseases and can act as modulators of the immune response in macrophages (Wang and Mazza, 2002).

Hom Nil rice and black glutinous rice are become popular among Thai consumers. Concerning public food safety, Hom Nil rice and black glutinous rice in this study were mutagenic after incubating with sodium nitrite; therefore, it might be the possible risk of gastric cancer in consumers that simultaneously consume nitrite containing foods. Nevertheless, the mutagenicity of nitrite-treated standard mutagens was inhibited by the rice extracts; the effect makes such rice potentially useful in dietary chemoprevention.