

CHAPTER V



DISCUSSION

Attempts in the present experiment were made to simulate the events which may occur during consumption of a meal consisting of cooked semi-processed instant noodles and released nitrite from other food item. The extracts from noodles cooked with seasonings, noodles only and seasonings only were treated with excess nitrite at pH 3-3.5 to imitate the phenomenon occurred during acid digestion of the stomach. The finding in this experiment that all nitrite treated extracts from noodle, seasoning and noodle cooked with seasoning are direct mutagens suggested that the samples contain some precursors readily interact with nitrite. Many investigators (Marquard *et al.*, 1977; Piacek-Llanes and Tannenbaum, 1982; Wakabayashi *et al.*, 1984) suggested that direct-acting mutagens/carcinogens formed from nitrite and the precursors of mutagens in the acid conditions of the stomach were possible candidates for the causation of human gastric cancer.

5.1 Mutagenicity of Extracts from Noodle

The results of this study show that the nitrite treated extracts from all types of noodles were higher mutagenic on *S. typhimurium* on TA 98 than those on TA 100. During processing noodle was sprayed with chicken extract (personal communication) on the surface of noodle before frying. It is known that meat flavour contained mutagenic heterocyclic amines classified as Aminoimidazoazaarenes (IQ) and Carboline (non-IQ) (Solyakov *et al.*, 1999). Heterocyclic amines show higher mutagenicity in *S. typhimurium* TA 98, a frameshift mutation detector, than in *S.*

typhimurium TA 100, a base-pair change mutation detector. Sasagawa *et al.* (1988) found that the amino group of IQ-type heterocyclic amines was converted to a nitro group if it was incubated with 50 mM sodium nitrite and the resulting compound showed a similar mutagenicity to the original compounds in the absence of activating system which was similar to the result of the present study. Nitro-IQ was also found to induce somatic mutation in *Drosophila melanogaster* (IARC, 1993). Moreover, mutagenicity of the nitrite treated extract from noodle may be due to the starch itself. Since Friedman *et al.* (1990) found that heated blends of 80% gluten with 20% amylose extract were weakly mutagenic without microsomal activation in strain TA 98. However, there is no information on the effect of nitrite on such heated sample in this study.

5.2 Mutagenicity of Extracts from Seasoning

It was speculated that meat flavours used in the instant noodle may prepared from heated vegetable or meat extract. However, it was found that no mutagenic activity was seen in stock cubes prepared from vegetable produce (Dolara and Bianchini, 1988) and no heterocyclic amines was detected in such processed flavouring based on hydrolysed plant proteins (Gross, Gruter and Heyland, 1992). Therefore, seasoning of each instant noodle in the present study should be prepared from meat soup stock mix with spices and other salts and was supposed to contain some heterocyclic amines. Solyakov *et al.* (1999) stated that processed flavours produced by heating a complex mixture of different ingredients for several hours contained a series of heterocyclic amines since some precursors of heterocyclic amines occurred naturally in many of the ingredients used.

The mutagenicity of product of nitrite treated Pa-lo duck flavour seasoning seemed to be higher than that of most samples on *S. typhimurium* TA 100. Considering on the ingredients of such seasoning, it was found that they were caramel, duck powder, star anise powder and cinnamon powder. Tongyonk (2001) found that after treatment of the extract from star anise powder and cinnamon with excess nitrite, they were direct mutagenic towards both strains of *S. typhimurium*. Caramel was also contribute to the direct mutagenicity of such flavour since Ishidate *et al.* (1984) found that caramel was weakly positive and induced 195 revertants (81 in control) in TA 100 at 50 mg/plate without activating system.

Mutagenicity of Sour Shrimp flavour treated with nitrite was not surprised. It was found that flavour composed of dried shrimp also the source of mutagen precursor for nitrite treatment. Mende, Spiegelhalder and Preussmann (1991) found that dried shrimp from Thailand contained about 150 ppb of N-nitroso-N-methylurea after treatment with 100 mM nitrite pH 1 at 37 °C for 1 h.

This study indicated that the non-enzymatic formation of direct-acting mutagens such as nitrite treated products of indirect acting mutagens is physiologically important, especially with regard to the etiology of gastric malignancies. Consequently mutagenic substances should be prevented from human diet.

5.3 Mutagenicity of Extracts from Noodle Cooked with Seasoning

Calculating number of revertants obtained based on the original weight of sample, namely noodle and seasoning per package, it was found that number of revertants of each nitrite treated extract from noodle cooked with seasoning was not an ordinary summation of those obtained from the value of noodle alone and that of

seasoning alone. It was also found that expected value from noodle cooked with seasoning higher than actual value. It was speculated that noodle cooked with seasoning might had less precursors for the interaction with nitrite. The finding is supported by Yu *et al* (2001) who found that refined wheat inhibited the mutagenic activities of 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) and 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx).

5.4 Specific Mutagenic Activity

From these results, it was found that specific mutagenic activity of seasoning per gram showed the highest when comparing noodle and noodle cooked with seasoning. It is not surprised since most seasonings contained some precursors such as meat flavour, spices and other components (such as caramel) which exhibited direct mutagenicity after being interacted with nitrite. It was found that ingredients of seasoning composed of meat flavour containing 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) (Solyakov *et al.*, 1999), spice (Gry, 1995) and other components (such as caramel) were mutagenicity after being interacted with nitrite.

Implication to Consumer

According to the results obtained from this study, it was found that the consumer should not consume instant noodle with nitrite containing food (such as kun-chiang, sausage, bacon, ham etc). It was suspected that two-thirds of nitrite entering the average stomach originated in saliva and slightly less than one-third comes from cured meats (White, 1975). The consumer should fill various vegetables, eggs, fresh meat into instant noodle. Many studies have examined the inhibition of heterocyclic amines mutagenicity in the Ames test by vegetable extracts such as vitamin C, chlorophyll etc (Kada, Morita and Inoue, 1978) and specific dietary components such as anthraflavic acid, chlorophyllin, vitamin A etc (Arimoto *et al.*, 1980; Busk, Ahlborg and Albanus, 1982; Hayatsu *et al.*, 1981; Hayatsu Arimoto and Negishi, 1988; Ayrton, Ioannides and Walker, 1988; Dashwood, Breinholt and Bailey, 1991). Many fruits and vegetables were shown to reduce the mutagenic activities of IQ, MeIQ and MeIQx *in vitro* (Edenharder, Leopold and Kries, 1995). Dietary fiber, glycosides and chlorophyll were suggested to be the source of the antimutagenic behavior (Edenharder *et al.*, 1995).

Some studies indicate that heterocyclic amines bind to dietary fiber *in vitro* (Sjodin *et al.*, 1985; Vikse, Balsrud Mjelva and Klurgsoyr, 1992). In addition, biogenic amines, namely tryptamine, 5-hydroxy-tryptamine and tyramine, and plant flavonoids reduced the *in vitro* mutagenic activity of IQ, MeIQ, MeIQx, Trp-P-1 and Trp-P-2 according to the Ames/*Salmonella* assay (Alldrick, Flynn and Rowland, 1986).

Since it was found that both the noodle and the seasoning in this study contained mutagen precursor readily interact with nitrite to form direct mutagens. It is suggested to consume less seasoning as possible or use homemade fresh meat stock

instead. In addition, it is advised to rinse the noodle with hot water before cooking. Further study on inhibiting the formation of such precursor during processing of both components of the instant noodle should come into attention of the producers.