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EFFECTS OF MAILLARD-TYPE PRODUCTS ON SERUM ENZYMES IN RATS AND ON MUTAGENICITY IN *SALMONELLA TYPHIMURIUM*

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ABSTRACT: *The Maillard reaction products between amino acids and sugars are used effectively as flavors for processed foods and tobacco. Recently, considerable attention has been focused on the toxicological effects of Maillard browned compounds. Therefore, we have tested the safety on the three-types of Maillard products (KG-19, KG-24 and KG-32) prepared from this Research Institute. Throughout the observation period of the acute toxicity study in rats and the mutagenicity assay using Salmonella typhimurium (TA98, TA100), the test articles did not show any significant toxic or mutagenic signs.*

INTRODUCTION

The Maillard reaction appears to be the one to occur most commonly during browning and is accompanied by changes of aroma, loss of nutritional value, and development of antioxidative activity (Lingert *et al.*, 1983). It was found that Maillard reaction products between amino acids and sugars were effectives as flavors for processed food and cigarettes (Tsen *et al.*, 1983; Barbee *et al.*, 1983; Dworschak *et al.*, 1983; Milton, 1983). Therefore, the food or tobacco industry often employs the reaction to produce desirable aroma, colors and flavors (James and Bernard, 1983). Control of flavor and aroma in processed foods and tobacco is of the utmost importance in determining the quality of the finished products. The degree of Maillard reaction is estimated as extent of brown color and aroma produced (Eichner and Wolf, 1983).

Thus, the role of Maillard reaction, known as non-enzymatic browning reactions between amino acids and sugars is now accepted as one of the most important processes in flavor production. However, the Maillard reaction has been proposed to be involved in the formation of mutagenic activity in the meat crust during frying or broiling. In recent years, considerable attention has been focused on the toxicological effects of Maillard browned compounds (Stephen *et al.*, 1983: Nagao *et al.*, 1983: Hirohosa *et al.*, 1983). For the reason, the Maillard browning compounds used in processed tobacco to produce characteristic flavors were chosen for toxicity test. Three types of flavors formed from non-enzymatic browning reaction by different conditions with amino acids and sugar were administered into the rats for the acute toxicity study. The toxicity

observations were physiological signs, changes of rectal temperature, body weight, and activities of various serum enzymes. We also tested mutagenicity of the compounds using the *Salmonella typhimurium* strains. Also, the produced major compounds from Maillard reaction were identified by GC.

MATERIALS AND METHODS

Chemicals;

Sodium pyruvate, pyridoxal 5-phosphate, urease and NADH were purchased from Sigma Chemicals Co., 4-nitrophenyl phosphate, α-ketoglutaric acid, alanine and creatinine from Merck, salicylic acid and picric acid from Fluka, and other chemicals of highest quality grade available were obtained from various local commercial sources.

Preparations of flavors;

As a typical model of the Maillard reaction, mixtures of various amino acids (DL-alanine, glycine and DL-2-aminobutyric acid) and glucose in propylene glycol were heated at 120°C for 4 hr under reflux. The flavors, three browning reaction products, were obtained from mixtures with different composition of amino acids and glucose under the identical conditions. The Maillard-type compounds, three types of flavors, were named as KG-19, KG-24 and KG-32, respectively. For the purpose of this study, the test articles produced were stored at 4°C

Identification of components produced;

The major components of the produced flavor by the Maillard reaction were identified under GC conditions as follows.

GC condition

Sample	; 0.4u*l*	Column	; Supelco WAX-10, 0.25mm, 60m
Carrier gas	; N_2		
Inlet press	; 1.7Kg/cm^2	Temperature	; 70°C to 220°C
Instrument	; HP5730-A	Injection temp.	; 250°C
Attenuation	; 32 × 10	Program rate	; 3°C/min
Detector	; FID	Detector temp.	; 250°C for

Animal treatment;

We used male and female Sprague-Dawley rats(150-160g) obtained from animal breeding laboratory of this research institute. Equal number of each sex was used for each dose level and the females were nulliparous and nonpregnant. All animals were randomly selected and each gorup (five rats per cange) was housed in polycarbonate cages on soft-wood shavings. The flavors (KG-19, KG-24 and KG-32) were dissolved

in isotonic saline at 3, 6, and 12mg/0.2ml, respectively, and the dilutions were prepared on the day of the test. After single administration of a flavor into the rats, we had observed rats for 14 days. Whole bloods were obtained by cardiac puncture from these rats anesthetized with thiopental.

Serum preparation;

Whole blood collected from rats was allowed to clot at 4°C for 30 min. The serum was then separated from the clot by centrifugation of 3000 rpm at room temperature for 10min. Aliquots of the serum were then refrigerated fro later determination of various enzymes.

Assay of serum enzyme activities;

The activities of lactic acid dehydrogenase (LDH, Park *et al.*, 1982), glutamic-pyruvic transaminase (GPT, Reitman and Frankel, 1974A), glutamic-oxalacetic transaminase (GOT, Reitman and Frankel, 1974B), and alkaline phosphatase (AP, Walter and Schutt, 1974) were measured from the rat serum. Serum creatinine (White *et al.*, 1970A) and blood urea nitrogen (BUN, White *et al.*, 1970B) were also determined by spectrophotometry.

The changes in rectal temperature and body weight, and physiological signs such as mortality, convulsion, diarrhea etc. were observed. Rectal temperature of rats was measured with a thermometer inserted to a depth 1cm into anus after the injection of Maillard compounds, flavors, respectively. The temperature was read at 30 seconds after insertion into the anus. On the seventh (7th) and fourteenth (14th) day, body weights were recorded.

Mutagenesis assay;

Tester strains TA98 and TA100 of *Salmonella typhimurium* (Kind gift of Dr. Roh Jung Koo, Korea Research Institute of Chemical Technology) were used for the mutagenicity test of the flavors. The mutagenic activity of the test substances was evaluated over a wide range of concentration in *Salmonella typhimurium* both with and without the addition of a mammalian metabolic activation system, so-called S-9 mixture (Ames *et al.*, 1975; Maron and Ames, 1983).

RESULTS AND DISCUSSION

The products of Maillard-type browning reactions between amino acids and sugars have been added to cigarettes or processed foods to obtain certain aroma and flavors. Thus, the Maillard compounds, namely the flavoring substances used in tobacco or food additives, have been produced for many years. Recently, several reports have, however, demonstrated that these substances cause general toxicity and also mutagenicity in Ames test(Stephen *et al.*, 1983; Nagao *et al.*, 1983; Hirohisa *et al.*, 1983). Therefore, three kinds of flavors produced from different compositions ratio of

Table 1. Major Compounds produced from Maillard reaction*

Cis-3-hexanol	Furfuryl alcohol
2,5-dimethylpyrazine	5-methyl furfuryl alcohol
Tigialdehyde	Methyl cyclopentenolone
2,3,5-trimethylpyrazine	2-methyl-3-furyl acrolein
3-ethyl-2,5-dimethylpyrazine	2-acetyl pyrrole
2-acetyl furan	meta-cresol
5-methyl-2-furfural	2-formyl-5-methyl pyrrole
2-acethyl-1-ethyl pyrole	5-hydroxy methyl-2-furfural
2-acetyl-1-methyl pyrole	

*These compounds were found in KG-19 and same compounds were also identified in KG-24 or KG-32.

the mixtures made of amino acids with sugar were examined for the acute toxicity study.

The major compounds of these flavors had been identified by GC. As shown in Table 1, the major identified products were pyrazines, furans, furfurals, and methyl or acetyl derivatives of pyrrole. The above compounds were commonly found in KG-19, KG-24 and KG-32.

Rats were widely used for evaluating the hepatic toxicity of various compounds, and serum GPT is determined as one of the most common clinical index of liver injury (Kozma *et al.*, 1969; Lazar, 1974). The serum GOT activity is also used as a diagnostic enzyme for the myocardial diseases (Cook *et al.*, 1974). As shown in Table 2, the rats of each group were examined at approximately 0.5, 1, 2, 4, 6 and 8 hour, and daily for fourteen days for pharmacological or toxicological effects after dosing with three kinds of flavors, respectively. The activities of GOT and GPT showed no significant changes when compared with those of the control rats during the observation period of fourteen days after treatment with different concentrations (3, 6, 12mg) of KG-19.

LDH is generally found in high concentrations in kidney and liver etc. (Loegering, 1974; Marmo *et al.*, 1973). However, the serum activity of LDH values of normal rat vary widely. In this study, the serum LDH activities did not significantly change when compared to those of control rats during the observation period. Similar observations had been made for the same enzymes of rat sera after treatment with other flavors, namely KG-24, and KG-32, respectively. The activity of AP determined from rat serum had been compared with that of control serum after the i.p. administration of three-types of the Maillard compounds. A little decrease in activity of this enzyme was observed in male rats at 2 hr after injection of different concentrations of KG-19. In contrast to the result of this observation, the enzyme activity in female rats were increased a little at 1hr after the administration of the same substances. Similar results were also obtained from other flavors, KG-24 and KG-32 under the same conditions. Further studies of such difference are needed to evaluate the pharmacological and

Table 2. Changes of Serum Enzymes in Male (Female) Rats after Treatment of Flavor, KG-19*

Observation Period	GPT(u moles/1 of serum)	GOT (u moles/^{1} of serum)	LDH (u moles/min/dl of serum)	AP (u moles/min/l of serum)
Normal	13.2 ± 1.1**(16.41.0)**	57.4 ± 6.0 (42.9± 3.1)	44.3 ± 0.5 (42.8 ± 0.7)	313.6 ± 50.3 (247.0 ± 33.7)
0.5H	14.7 ± 1.9 (18.0 ± 1.2)	54.0 ± 5.4 (45.8 ± 4.2)	43.7 ± 4.2 (44.2 ± 4.3)	325.6 ± 50.5 (265.2 ± 35.3)
1	12.0 ± 1.5 (19.0 ± 1.3)	44.0 ± 3.9 (47.3 ± 6.7)	40.7 ± 3.9 (45.5 ± 5.7)	290.5 ± 41.7 (290.0 ± 35.8)
2	13.5 ± 2.3 (16.5 ± 0.9)	56.4 ± 5.7 (43.4 ± 3.6)	46.8 ± 3.0 (42.5 ± 5.4)	260.8 ± 25.7 (265.2 ± 40.8)
4	12.0 ± 1.8 (18.5 ± 1.6)	57.0 ± 7.0 (43.0 ± 6.3)	46.3 ± 2.1 (42.5 ± 4.6)	340.5 ± 47.2 (250.2 ± 40.7)
8	16.7 ± 2.6 (17.3 ± 1.1)	53.5 ± 6.2 (50.1 ± 2.8)	44.2 ± 0.6 (43.1 ± 2.3)	375.3 ± 67.4 (257.3 ± 40.5)
3 Day	17.0 ± 1.8 (15.7 ± 1.8)	53.7 ± 5.6 (48.5 ± 2.7)	45.5 ± 0.7 (43.0 ± 1.8)	350.1 ± 50.7 (263.2 ± 32.8)
7	17.0 ± 1.2 (17.1 ± 0.5)	52.0 ± 2.8 (46.2 ± 1.9)	44.0 ± 0.3 (45.0 ± 3.9)	370.4 ± 67.6 (255.8 ± 38.4)
14	17.0 ± 2.5 (15.9 ± 1.0)	55.0 ± 5.7 (41.7 ± 5.2)	45.0 ± 0.7 (42.7 ± 1.3)	345.1 ± 50.3 (252.8 ± 51.9)

*Dose level: 12mg of KG-19

**Mean ± Standard deviation of the mean

Table 3. Changes of Serum Creatinine and BUN Content after treatment of Flavor, KG-19*

Observation Period	Creatinine(mg/dl of serum)	BUN (mg/dl of serum)
Normal	0.51 ± 0.04** (0.50 ± 0.08)**	29.7 ± 6.9 (19.9 ± 3.5)
0.5H	0.42 ± 0.05 (0.43 ± 0.03)	23.1 ± 6.4 (17.5 ± 2.5)
1	0.40 ± 0.05 (0.42 ± 0.08)	19.7 ± 4.3 (18.5 ± 2.6)
2	0.41 ± 0.05 (0.45 ± 0.05)	18.9 ± 6.7 (19.2 ± 3.3)
4	0.46 ± 0.03 (0.50 ± 0.05)	23.9 ± 3.3 (24.0 ± 2.5)
8	0.46 ± 0.07 (0.48 ± 0.02)	26.4 ± 4.2 (20.8 ± 1.7)
3 Day	0.44 ± 0.05 (0.48 ± 0.05)	27.2 ± 7.1 (25.4 ± 3.1)
7	0.49 ± 0.08 (0.45 ± 0.07)	25.9 ± 3.7 (22.5 ± 4.2)
14	0.41 ± 0.04 (0.52 ± 0.05)	31.5 ± 5.2 (27.3 ± 2.0)

*Dose level is 12mg of KG-19
**Mean ± standard deviation of the mean

toxicological implication of this results. However, the normal value of AP activity in rat serum was a little higher in male than in female, and this result was in agreement with the reported values (Nakaue *et al.*, 1973; Schwartz *et al.*, 1973). Then, no toxicological signs were generally observed in male and female rats during the observation period of fourtheen days.

BUN concentration is a useful measure of kidney dysfunction, and rises significantly in the rat following kidney damage by foreign compounds. In the rat, BUN value does not vary with age or sex(Kozma *et al.*, 1969; Vondruska and Greco, 1973). No change of BUN content of rats had been found as shown in Table 3 as compared with those of the controls after administration of the same flavors. On the other hand, creatinine is the major waste product of some amino acid metabolism in muscle (Daniel and Lyubrica, 1979). Thus, free creatinine appears in the serum of blood, and the changes of creatinine content in serum could be used as a marker for kidney function test. The serum creatinine values had no significant change after the injection of KG-19. As with the BUN, both flavors tested did not have any effect.

In addition to the above observations, body weights of rats on the seventh and fourteenth day were recorded and the rectal temperature was observed daily. The body weights of experimental rats were increased normally. The rectal temperature, was about 38.5°C and all the rats tested were normal during the observation period. The same results were also obtained from rats treated with KG-24 and KG-32. Three flavors examined for the acute toxicity study, namely KG-19, KG-24 and KG-32, and they were generally observed to have no signs throughout 14 days of this work. However, further chronic toxicity study is necessary to identify safety of these flavors.

On the other hand, the test articles (or flavors) were evaluated for the mutagenicity in strains TA98 and TA100 of *Salmonella typhimurium* with and without the rat liver fraction at dose levels of 20, 50, 100, 200, 300 and 500 ug/plate (Fig. 1). The

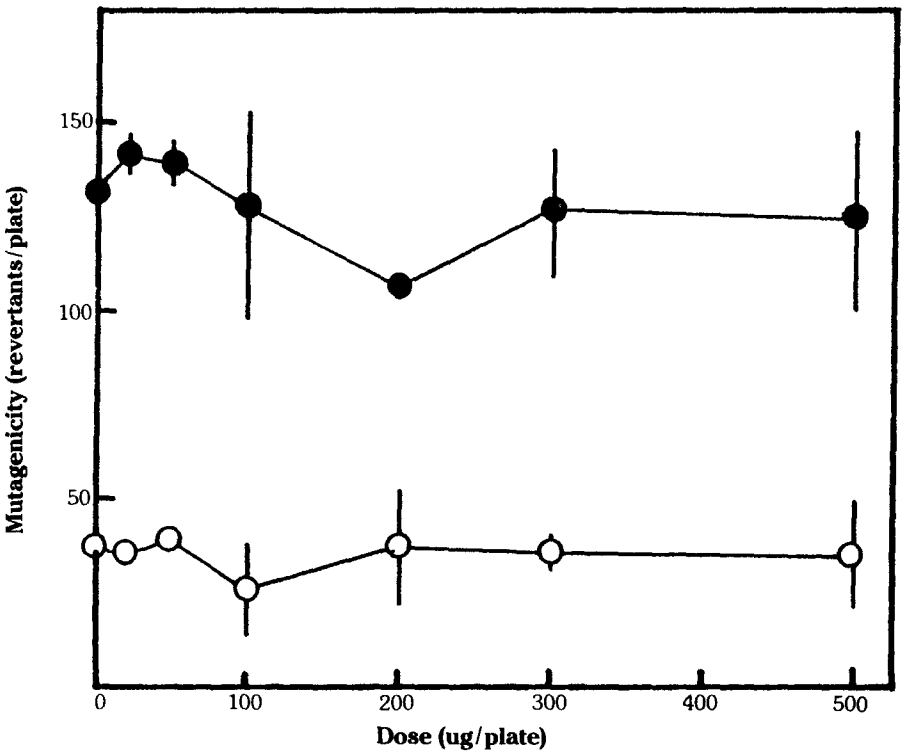

Fig. 1. Dose-response curve of mutaganicity by the Maillard reaction product, KG-19, with (○—○) TA98 and with (●—●) TA100

Table 4. The mutagenic activities of Maillard reaction products (500ug) on *Salmonella typhimurium* TA98 and TA100.

Sample	Revertants per plate	
	TA98	TA100
Spontaneous	37	103
KG-32	42	131
KG-24	66	154
KG-19	36	91

rat liver metabolic activation preparation contained 0.1ml of the S-9 supernatant (42.4mg protein per ml) per 1.0ml of S-9 mixture. The mutagenicity of these compounds, three-type flavors, was clearly negative in the two *Salmonella* tester strains, respectively (Table 4, Fig. 1). A positive result was defined as about 2.5-fold increase of spontaneous revertant colonies. Similar negative results were also obtained with KG-24 and KG-32.

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