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Estimation of Korean Adult's Daily Intake of Ethyl Carbamate through Korean Commercial Alcoholic Beverages Based on the Monitoring

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Abstract Levels of ethyl carbamate, by-product produced naturally during fermentation, in Korean alcoholic beverages were determined by Gas Chromatography/mass spectrometry/selected ion mode (GC/MS/SIM), and their daily intake by Korean adult group was estimated. In GC/MS/SIM analysis 0.8-10.1, 0.5-0.8, 0.4-0.9, 3.5-689.9, 8.4-30.3, 13.9-30.0, and 1.7-11.7 ppb of ethyl carbamate were detected in *soju*, beer, *takju*, fruit wine, *cheongju*, whiskey, and grape wine, respectively. Maximum daily exposure of ethyl carbamate through alcoholic beverage consumption was 7.41 ng/kg body weight/day for average Korean male, with one *soju* brand and two fruit wine brands showing high ethyl carbamate level.

Keywords: ethyl carbamate, urethane, alcoholic beverage, EDI

Introduction

Ethyl carbamate (Urethane: $NH_2COOCH_2CH_3$), which occurs naturally in most fermented foods and beverages, such as distilled spirits, wine, sake, whisky, *kimchi*, soy sauce, natto, yogurt, cheese, and bread (1-8), is known to be carcinogenic to a number of species including mice, rats, hamsters, and monkeys, suggesting a potentially carcinogenic risk to humans. Various methods for the analysis of ethyl carbamate have been performed (9-17). Based on experimental and epidemiological data, ethyl carbamate has also been classified as a possible human carcinogen (class 2B) by the International Agency for Research on Cancer (IARC, 1987) (3). Acute exposure to high level of ethyl carbamate may cause injury to various organs, especially liver, central nervous system, and hemopoietic system. Rodents exposed to ethyl carbamate by inhalation or ingestion show incidences of lung tumors. However, although ethyl carbamate is a probable human carcinogen based on sufficient animal data, no human evidences are yet available. Previous reports have suggested that the carcinogenicity of ethyl carbamate appears to be mediated through a metabolic pathway involving sequential cytochrome P-450 and turn into vinyl carbamate and vinyl carbamate epoxide, the latter of which reacts with DNA to yield DNA adducts (Fig. 1). This reaction is probably the mode of the mutagenic action observed in many cellular and animal systems (19-22).

The mechanisms of ethyl carbamate formation have been investigated in many studies (6, 7, 12, 14, 23). From these studies it can be deduced that the formation of ethyl carbamate is a spontaneous chemical reaction involving ethanol and a compound that contains a carbamyl group. The reported precursor of ethyl carbamate in fermented foods and beverages is believed to be urea, cyanate, carbamyl phosphate, or diethyl pyrocarbonate, an anti-microbial food additive. In certain alcoholic beverages, urea is the most important precursor of ethyl carbamate. In addition, L-arginine is one of the amino acids present in alcoholic beverage, and is catabolized by the yeast or lactic acid bacteria into ornithine, ammonia, and carbon dioxide during fermentation by means of the arginase enzyme (24-27). Urea is produced as an intermediate

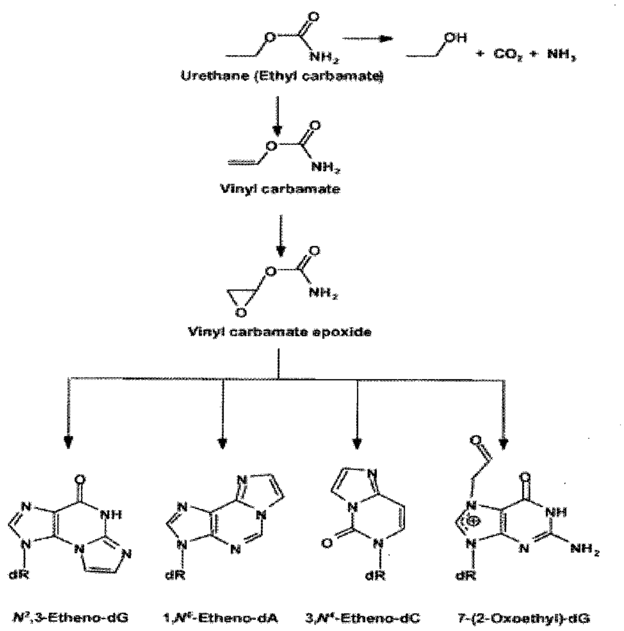

Fig. 1. Metabolic pathways and DNA adducts derived from urethane (ethyl carbamate). Similar adducts have been detected in RNA. The abbreviations used are dG, deoxyguanosine; dA, deoxyadenosine; dC, deoxycytidine; dR, deoxyribose (by Beland *et. al.* (18)).

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product, and the yeast secrets the excess urea into the environment, because it is a denaturing agent.

Levels of ethyl carbamate differ in alcoholic beverages differ depending on the processing and storage conditions (28). Some Sherries are baked and bourbons are distilled at high temperatures to provide a rich taste. Both baking and heating processes may raise ethyl carbamate level, because heating appears to accelerate the production of ethyl carbamate. Ethyl carbamate level also vary significantly, even among different bottles of the same type or brand (1).

In 1985, report by the Health and Welfare Canada, FDA's Canadian counterpart, of the presence of ethyl carbamate in distilled spirits and wines attracted international attention. The Canadian Health and Welfare Department established regulatory limits on the ethyl carbamate content at 30, 100, 150, and 400 ng/g for table wines, fortified wines (such as sherries and ports), distilled spirits, and fruit brandies and liqueurs, respectively (2, 4, 6, 11, 29). The wine and distilled spirit industries in United States also set voluntary limits on the ethyl carbamate contents for both domestic and imported alcoholic products and successfully decreased the contamination levels of the ethyl carbamate by educating the alcoholic beverage industry (2, 4, 29). For instance, U.S. FDA published "Preventative Action Manual" to reduce the risk of natural formation of ethyl carbamate in wine production by suggesting effective ways to minimize ethyl carbamate formation during fermentations.

However, in Korea, despite the popularity and high consumption of alcoholic beverages, there are currently no limits on the ethyl carbamate content in alcoholic beverages. Therefore, this study was conducted to determine the concentrations of ethyl carbamate in Korean alcoholic beverages, estimate daily exposure, and compare the results with the 'virtually safe dose' of Schlatter and Luz (30) and 'No Significant Risk Levels' of California State Government (31).

Materials and Methods

Sample collection *Soju*, beer, and *takju*, commercial alcoholic beverages in Korea, were selected for sampling, because they were previously included in 100 major Korean diets, representing 91.3% of total diets (33). Seven brands of *soju* and six brands of beer, representing more than 90% of the market share, were purchased at grocery stores, while seven brands of *takju* from seven major cities across Korea were purchased at traditional markets. Three samples of each brand of alcoholic beverages were collected at monthly intervals. In addition, fruit brandy, *cheongju*, whiskey, and grape wine were also sampled, and the major products of these alcoholic beverages were purchased to analyze the distribution of ethyl carbamate levels in Korean alcoholic beverages.

Gas chromatography/mass spectrometry detection Agilent 6890 gas chromatograph with a 5973N mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) was used for separation and determination of ethyl carbamate in the extracts. Determination of ethyl carbamate in each sample was triplicated and cross-checked again at Cooperate Center for Research Facility in Sungkyunkwan University to reduce analytical errors.

Gas chromatography conditions GC conditions were as follows: carrier gas flow, helium at 0.7 mL/min; injection volume, 2 μL ; column, i.d. 30 m × 0.25 mm, DB-INNOWAX capillary column; oven temperature, 1 min at 80°C, increase to 120°C at 5°C/min, and hold for 15 min, increase to 250°C at 20/min; and injector, 25°C.

Mass spectrometer conditions were as follows: ion source, 200°C: electron impact ionization potential at 70 eV; selected ion mode (SIM), m/z of 62, 74, and 89; and injection mode, splitless. Peaks containing all three ions (m/z; 62, 74, 89) were regarded as ethyl carbamate.

Ethyl carbamate standard solutions Stock solution (1 mg/mL) was prepared as follows. In a 100-mL volumetric flask, 100 mg ethyl carbamate (Sigma, USA) was dissolved in ethyl acetate, diluted, and mixed thoroughly. Subsequently, 100 μg/mL working standard solution was prepared by diluting stock solution (1 mg/mL) tenfold with ethyl acetate.

Sample preparation Sample clean-up procedure was conducted using the method of Conacher *et al.* (11) with some modification. Alcoholic beverage samples were adjusted to 10% of alcohol content with distilled water and mixed to maintain approximately same level of alcohol in all samples. Fifty grams of diluted samples were weighed into a 250-mL centrifuge bottle, and 30 g sodium chloride was added and swirled 1-2 min to saturate the solution. Seventy-five milliliters dichloromethane was added and vigorously shaked for 1 min. The solution was centrifuged at 2,000×g until phase separation occurred. Dichloromethane layer was drawn off using a suitable suction device. The remnant was filtered into a 500-mL evaporator flask through 40 g anhydrous sodium sulfate on Whatman No.1 filter paper in a conical glass funnel. The extraction step was repeated three more times. Three milliliters ethyl acetate was added to the dichloromethane extract, and concentrated in a rotary evaporator below 28°C at reduced pressure until volume decreased to 1 mL. Dichloromethane was completely removed at this step, and the extract was not evaporated to dryness or to near dryness to minimize ethyl carbamate loss. The liquid was transfered to a 2-mL vial, and the flask was rinsed with 1 mL ethyl acetate and mixed well. The mixture (2 μL) was injected into gas chromatograph for analysis.

Results and Discussion

Ethyl carbamate levels in Korean alcoholic beverages Recently, GC/MS SIM analysis has been the most widely used method due to its low detection limit. Park and Lee (16) reported 0.3 ppb of detection limit for ethyl carbamate analysis with GC/MS SIM. Recovery tests for ethyl carbamate in various kinds of alcoholic beverages were conducted in analytical method-developing studies (4, 9, 11, 12, 16). According to these studies, the recoveries of ethyl carbamate from distilled liquor, beer, brandy, sherry, wine, and sake were 91.8, 94.3, 85.0, 83.8, 85.3, and 105.1%, respectively.

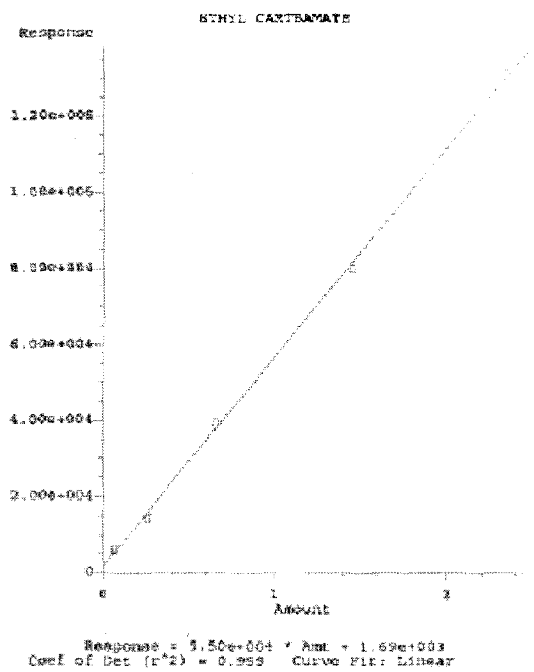

Fig. 2. Calibration curve for GC/MS analysis of ethyl carbamate.

GC/MS SIM analysis provided a calibration curve with R^2=0.999 (Fig. 2), with which the concentration of ethyl carbamate in alcoholic beverages were determined. Ethyl carbamate contents in 45 brands of 8 types Korean alcoholic beverages ranged from 0.4 to 689.9 ppb (Table 1). The ethyl carbamate contents were 0.8-10.1 ppb in *soju*, 0.5-0.8 ppb in beer, 0.4-0.9 ppb in *takju*, 3.5-689.9 ppb in fruit brandy, 13.9-30.0 ppb in whisky, 8.4-30.3 ppb in *cheongju*, and 1.7-11.7 ppb in grape wine. Ethyl carbamate contents of *soju* were relatively high and varied greatly depending on the brands. Considering the fact that *soju* companies are supplied with the same spirits, the differences in the amounts of ethyl carbamate contained in each *soju* brand was seemingly due to differences in the manufacturing processes and storage conditions among the *soju* companies, implying that the ethyl carbamate content in *soju* can be reduced by modifying such manufacturing processes and storage conditions. Although the ethyl carbamate content in *soju* was not particularly high, high consumption of *soju* among Koreans shows it can be an important source of ethyl carbamate. The ethyl carbamate content of beer ranged from 0.3 to 0.8 ppb, which was much lower than the finding (10 ppb) of Battaglia *et al.* (1).

The highest average content of ethyl carbamate in the samples was found in the fruit bandy, particularly stone fruit brandies made of plum, while that of beers was lower. As reviewed by Battaglia *et al.* (1), who also found the highest ethyl carbamate content in stone-fruit brandies (100-20,000 ppb), the ethyl carbamate content in alcoholic beverages varied greatly depending on the beverage types.

Table 1. Contents of ethyl carbamate in alcoholic beverages

Items	No. of brands	Mean alcohol content (%)	Ethyl carbamate levels (ppb)	
			Range	Mean
Soju	7	21.0	0.8-10.1	3.0
Beer	6	4.6	0. 5-0.8	0.5
Takju	7	6.0	0.4-0.9	0.6
Fruit brandy	7	14.0	3.5-689.9	196.7
Whisky	5	40.0	13.9-30.0	20.1
Cheongju	5	14.0	8.4-30.3	20.2
Wine	3	12	1.7-11.7	4.9

Exposure estimation The average daily intakes of alcoholic beverages and the resulting ethyl carbamate intakes are shown in Table 2 and 3. The corresponding mean and maximum levels of estimated ethyl carbamate intake of an average Korean per day are also listed. The estimated daily intakes (EDI) of ethyl carbamate through alcoholic beverages were calculated by multiplying the maximum and minimum consumptions with the mean ethyl carbamate content. Although the daily intake of *soju* was not as high as that of beer (Table 2), the high content of ethyl carbamate in *soju* contributed significantly to the total intake of ethyl carbamate. Conversely, although beer had the highest consumption, its lower ethyl carbamate content meant that it contributed less to the total intake of ethyl carbamate. The highest EDI was 2.90 ng/kg b.w./day through *soju*. Although ethyl carbamate content in fruit brandy was much higher than those of the other beverage samples, its contribution to EDI was less than *soju* due to its low consumption. The EDI was only based on the ethyl carbamate levels found in the alcoholic beverages

Table 2. Intakes of alcoholic beverages (g/day)

Items	Mean	Maximum	Minimum
Soju	27.6	57.9	2.7
Beer	37.1	80.6	5.3
Takju	7.3	20.6	2.5
Fruit brandy	0.3	0.8	0.2
Whisky	0.5	1.1	0.1
Cheongju	0.5	0.7	0.1
Wine	0.4	0.9	0.1

Table 3. Estimated daily intakes (EDI) of ethyl carbamate by ingestion of alcoholic beverages (ng/kg body weight/day)

Items	Mean	Maximum	Minimum
Soju	1.38	2.90	0.14
Beer	0.33	0.67	0.04
Takju	0.07	0.21	0.03
Fruit brandy	1.09	2.62	0.66
Whisky	0.18	0.37	0.03
Cheongju	0.17	0.24	0.03
Wine	0.03	0.07	0.01
Total	3.25	7.41	0.94

analyzed in this study. Thus, if more brands and samples from different regions with distinct manufacturing processes and storage conditions were analyzed, the estimated ethyl carbamate exposure could be refined.

The 'virtually safe dose' for a lifetime risk level of 10^{-6} was calculated to be between 20 and 80 ng/kg body weight using the estimation by Schlatter and Luts (30). However, the California State Government used a 'no significant risk level' of 0.7 μg/day of ethyl carbamate in California Proposition 65 (31). As such, the accepted daily intake of ethyl carbamate has not yet been well defined. Exposure to ethyl carbamate at the levels estimated above is grouped into the 'no significant risk level', which is lower than the 'virtually safe dose'. However, in the case of consuming certain brands of the alcoholic beverages with high ethyl carbamate contents, the maximum intakes of ethyl carbamate may be much higher than 7.41 ng/kg body weight/day. In addition, this value is only based on the Korean popular alcoholic beverages and excludes fermented foods, such as *kimchi*, soy sauce, yogurt, and soy paste, which are highly suspected to contain ethyl carbamate; the per capita consumption levels of these fermented foods are not negligible in Korea (34). Therefore, total intake of ethyl carbamate by Koreans through not only alcoholic beverages but also fermented foods has high possibility to be higher than the 'no significant risk level' of 0.7 μg/day. Nonetheless, the present results reveal that alcoholic beverages represent a significant part of the ethyl carbamate intake. Therefore, the alcoholic beverage-producing industries need to make efforts to reduce the amount of ethyl carbamate in their products. Should the ethyl carbamate contents in one sample of *soju* and two samples of plum wine were reduced to the average contents found in the other *soju* brands, EDI of ethyl carbamate from alcoholic beverages would be reduced by 50%.

Identification of the mechanism involved in the ethyl carbamate formation during fermentation and the effects of processing and storage conditions on the formation are important to achieve reduction of ethyl carbamate in foods. Regulations and nation-wide monitoring of ethyl carbamate levels in fermented foods should also be implemented to protect the health of the consumers. In this study we determined the concentrations of ethyl carbamate in major Korean commercial alcoholic beverages, which were expected to contribute highly to the intake of ethyl carbamate in Korean adults. In addition, studies on the monitoring of ethyl carbamate in Korean major fermented foods, such as *kimchi*, soy sauce, yogurt, and soy paste, are currently underway to establish more accurate and comprehensive exposure and risk assessments of ethyl carbamate.

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References

1. Battaglia R, Conacher HBS, Page BD. Ethyl carbamate (urethane) in alcoholic beverages and foods (abstract). Food Addit. Contam. 4: 477-496 (1990)
2. Canas BJ, Harvey DC, Robinson LR, Sullivan MP, Joe Jr. FL, Diachenko GW. Ethyl carbamate levels in selected foods and beverages. J. AOAC Int. 72: 873-876 (1989)
3. Mah JH, Yoon MY, Cha GS, Byun MW, Hwang HJ. Influence of curing and heating on formation of N-nitrosamines from biogenic amines in food model system using Korean traditional fermented fish product. Food Sci. Biotechnol. 14: 168-170 (2005)
4. Hasegawa Y, Nakamura T, Tonogai Y, Terasawa S, Ito Y, Chiyama M. Determination of ethyl carbamate in various fermented foods by selected ion monitoring. J. Food Protect. 53: 1058-1061 (1990)
5. Koh EM, Kwon HJ. Determination of specific carcinogen, ethyl carbamate, in kimchi. Korean J. Food Sci. Technol. 28: 421-429 (1996)
6. Matsudo T, Aoki K, Fokuta N, Sasaki M, Uchida K. Determination of ethyl carbamate in soy sauce and its possible precursor. J. Agr. Food Chem. 41: 352-356 (1993)
7. Ough CS. Ethyl carbamate in fermented beverages and food. I. Naturally occuring ethyl carbamate. J. Agr. Food Chem. 24: 323-328 (1976)
8. Uthurry CA, Varela F, Colomo B, Lepe JAS, Lombardero J, Garcia del HJR. Ethyl carbamate concentrations of typical Spanish red wines. Food Chem. 88: 329-336 (2004)
9. Breumley WC, Canas BJ, Perfetti GA, Mossoba MM, Sphon JA, Corneliussen PE. Quantitation of ethyl carbamate in whisky, sherry, port and wine by gas chromatography/tandem mass spectrometry using a triple quadruple mass spectrometer. Anal. Chem. 60: 975-978 (1988)
10. Cairns T, Siegmund EG, Luke MA, Doose GM. Residue levels of ethyl carbamate in wines and spirits by gas chromatography and mass spectrometry/mass spectrometry. Anal. Chem. 59: 2055-2059 (1987)
11. Conacher HBS, Page BD, Lau BPY, Lawrence JF, Bailey R, Calway P, Hanchay JP, Mori B. Capillary column gas chromatographic determination of ethyl carbamate in alcoholic beverages with confirmation by gas chromatography/mass spectrometry. J. AOAC Int. 70: 749-751 (1987)
12. Walker G, Winterlin W, Fouda H, Seiber J. Gas chromatographic analysis of urethane (ethyl carbamate) in wine. J. Agr. Food Chem. 22: 944-947 (1974)
13. Jagerdeo E, Dugar S, Foster GD, Schenck H. Analysis of ethyl carbamate in wines using solid-phase extraction and multidimensional gas chromatography/mass spectrometry. J. Agr. Food Chem. 50: 5797-5802 (2002)
14. Joe Jr. FL, Kline DA, Miletta EM, Roach JAG, Roseboro EI, Fazio T. Determination of urethane in wines by gas-liquid chromatography and its confirmation by mass spectrometry. J. AOAC Int. 60: 509-516 (1977)
15. Lachenmeier DW, Frank W, Kuballa T. Application of tandem mass spectrometry combined with gas chromatography to the routine analysis of ethyl carbamate in stone-fruit spirits. Rapid Commun. Mass Sp. 19: 108-112 (2004)
16. Park GB, Lee SG. Quantitative analysis of ethyl carbamate in Korean alcoholic beverages by chromatography with mass selective detection. Anal. Sci. 15: 26-30 (2002)
17. Ma YP, Deng FQ, Chen DZ, Sun SW. Determination of ethyl carbamate in alcoholic beverages by capillary multi-dimensional gas chromatography with thermionic specific detection. J. Chromatogr. A 695: 259-265 (1995)
18. Beland FA, Benson RW, Mellick PW, Kobatch RM, Roberts DW, Fang JI, Doerge DR. Effect of ethanol on the tumorigenicity of urethane (ethyl carbamate) in B6C3F mice. Food Chem. Toxicol. 43: 1-19 (2004)
19. Cha SW, Lee HJ, Cho MH, Lee MH, Koh WS, Han SS, Kim JA, Lee ES, Nam DH, Jeong TC. Role of corticosteron in ethyl carbamate-induced immunosuppression in female BALB/c mice. Toxicol. Lett. 119: 173-181 (2001)
20. Miller YE, Dwyer-Nield LD, Keith RL, Le M, Franklin WA, Malkinson AM. Induction of high incidence of lung tumors in C57BL/6 mice with multiple ethyl carbamate injections. Cancer Lett. 198: 139-144 (2003)
21. Sakano K, Oikawa S, Hiraku Y, Kawanishi S. Metabolism of

carcinogenic urethane to nitric oxide is involved in oxidative DNA damage. Free Radical Bio. Med. 33: 703-714 (2002)
22. Tomisawa M, Suemizzu H, Ohnishi Y, Maruyama C, Urano K, Usui T, Yasuhara K, Tamaoki N, Mitsumori K. Mutation analysis of vinyl carbamate or urethane induced lung tumors in ras H2 transgenic mice. Toxicol. Lett. 142: 111-117 (2003)
23. Ough CS. Ethyl carbamate in fermented beverages and foods . Possible formation of ethyl carbamate from diethyl dicarbonate addition to wine. J. Agr. Food Chem. 24: 328-331 (1976)
24. Arena ME, Saguir MC, Nadra M. Arginine, citrulline and ornithine metabolism by lactic acid bacteria. Int. J. Food Microbiol. 52: 155-161 (1999)
25. Mira de OR, Liu SQ, Patchett ML, Pilone GJ. Ethyl carbamate precursor citrulline formation from arginine degradation by malolactic wine lactic acid bacteria. FEMS Microbiol. Lett. 183: 31-35 (2000)
26. Tonon T, Lonvaud-Funel A. Arginine metabolism by wine *Lactobacilli* isolated from wine. Food Microbiol. 19: 451-461 (2002)
27. Uthurry CA, Varela F, Colomo B, Lepe JAS, Lombardero J, Garcia del HJR. Ethyl carbamate production by selected yeast and lactic acid bacteria in red wine. Food Chem. 94: 262-270 (2004)
28. Hasnip S, Caputi A, Crews C, Brereton P. Effects of storage time and temperature on the concentration of ethyl carbamate and its precursors in wine. Food Addit. Contam. 21: 1155-1161 (2004)
29. Foulke JE. Urethane in alcoholic beverages under investigation. U.S. FDA/CFSAN Consumer Report (1993)
30. Schlatter J, Lutz WK. The carcinogenic potential of ethyl carbamate (urethane): risk assessment at human dietary exposure levels. Food Chem. Toxicol. 28: 205-211 (1990)
31. California Environmental Protection Agency. Proposition 65 Status Report Safe Harbor Levels: No significant risk levels for carcinogens and maximum allowable dose levels for chemicals causing reproductive toxicity. California Environmental Protection Agency, USA (2004)
32. Zimmeli B, Schlatter J. Ethyl carbamate: analytical methodology, occurrence, formation, biological activity and risk assessment. Mutat. Res.-Rev. Mutat. 259: 325-350 (1991)
33. Ministry of Health and Welfare. Korean health and nutrition survey and the report. Ministry of Health and Welfare, Korea (2002)
34. Kim DH, Song HP, Choi ID, Kim BK, Cheong MY, Byun MW. Effect of the salt oncentration on the radiation sensitivity of some fermentative microorganisms inhabiting salted and fermented food. Food Sci. Biotechnol. 13: 387-389 (2004)