

Analysis of ethyl carbamate in plum wines produced in Korea

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Abstract Ethyl carbamate is naturally occurring compound, commonly found in many fermented foods and alcoholic beverages. During the process of plum wine production, ethyl carbamate can be formed. To this date, limited studies were conducted to monitor the ethyl carbamate in the plum wine brewed in-house. The objective of this study was to analyze the ethyl carbamates in plum wine, that were produced in differently: in-house and commercial production. A total of 33 plum wines were analyzed. The levels of ethyl carbamate ranged from N.D to 352.7 µg/kg in plum wines available in Korea. The current level of ethyl carbamate in plum wine was below the governmental regulation. However, continuous monitoring and further develop a strategy to reduce the level of ethyl carbamate in plum wine is in need, as the highest level of ethyl carbamate in plum wine is near the governmental standard (400 µg/kg).

Keywords Ethyl carbamate · GC/MS · Japanese apricot · Plum · Plum wine

Introduction

Japanese apricot (*Prunus mume* S. et Z.), native to China, belongs to the Rosaceae family, genus *Prunus* L., called mei in China [1]. Japanese apricot is famous for its ornamental value and producing healthy food [2]. It is mostly distributed in 10 provinces and areas of mainland China, including Yunnan, Guangdong, Zhejiang, Jiangsu, Guangxi, Fujian, as well as in Japan, Korea and Vietnam [2]. Another name of Japanese apricots is plum. Plum is classified as stone fruit category, as it has stone-like seed inside fruit. The fruits of plum are mostly used for food production, disease treatment [1, 3] and preventing cancer cell proliferation [4]. Plums are harvested around June in Korea. Upon harvest, fruits are utilized for further processing such as, plum pickles, plum extract and plum wine, due to its perishable nature [5]. In 2008, 28,251 tons of plum wine were produced in Korea, with 9% processed by food processing companies and about 91% (25,708 tons) were used by consumers for the production of drinks, wine, or pickles [6].

One of the naturally occurring chemical hazards associate with plum wine is ethyl carbamate. Manufacturing of plum wine involves fermentation of plum, which involves enzymatic degradation of stone to form cyanogenic glycosides (such as amygdalin). After the hydrolysis, cyanate generated from cyanogenic glycosides are further react with ethanol to form ethyl carbamate [7, 8] The potential toxicity of ethyl carbamate for human health has been reported previously [9], in that genotoxicity and carcinogenicity in many animal studies in mice, rats, hamsters, and monkeys, were reported steadily [10]. Recently, the International Agency for Research on Cancer (IARC) re-classified ethyl carbamate as a group 2A carcinogen, probably carcinogenic to humans [11]. The government regulations

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on ethyl carbamate in alcoholic beverage in different countries are listed in Table 1. The maximum allowable levels of ethyl carbamate in beverages were first set by Canadian government, as 30 µg/kg for wines, 100 µg/kg for fortified wines, 150 µg/kg for wine spirits, brandies, and whiskies, and 400 µg/kg for fruit brandies, cordials, and liqueurs [12].

The Food and Agriculture Organization of the United Nations/World Health Organization [13] has reviewed the levels of ethyl carbamate in alcoholic beverages and foods in various countries. A total of 190 brands of alcoholic beverages in UK and Denmark were collected and monitored the levels of ethyl carbamate. In their study, the level of ethyl carbamate in alcoholic beverages in UK and Denmark ranged from not detectable up to 12 µg/kg [14]. Among alcoholic beverages, ethyl carbamate in wine, sake, and whiskey were highly detected, especially in brandy and spirits made from stone fruits [9]. Foulk [15] reported 1200 µg/kg of ethyl carbamate in fruit brandies. The highest level of ethyl carbamate among 6004 alcoholic beverages, was found in Japanese sake with mean of 122 µg/kg [16]. In Korea, 65 liqueurs made from fruits, except plum wines, had ethyl carbamate levels below 30 µg/kg, while liqueurs made with plum (mainly plum wine) showed ethyl carbamate levels from 30 to 476 µg/kg [17]. Previous study on surveillance of ethyl carbamate in 95 alcoholic beverages available in Korea, that included takju, sake, yakju, fruit wine, shochu, liqueur, and brandy, reported that plum wine has the highest ethyl carbamate levels with 79.18 µg/kg [18]. Hong et al. [19] reported the ethyl carbamate in plum wine as 3.48–689.87 µg/kg.

To this date, there is no governmental regulation set for controlling of ethyl carbamate except for grape wine (30 µg/kg). Considering that the levels of ethyl carbamate was not systematically studied in plum wine produced in-house, it is crucial to assess the current level of ethyl carbamate in plum wine in order to provide the recommendations for ethyl carbamate regulation. The objective of this study was to analyze the ethyl carbamates in plum

wine, that were produced in differently: in-house and commercial production.

Materials and methods

Materials

Ethyl carbamate and butyl carbamate (an internal standard) were purchased from Sigma–Aldrich Inc. (St. Louis, MO, USA). Dichloromethane was purchased from J. T. Baker Chemical Co. (Phillipsburg, NJ, USA). Ethanol and acetone were purchased from Fisher Scientific (fisher chemical, USA). Diatomite solid-phase extraction column (Chem Elut column, volume, 50 mL) was obtained from Varian Co. (Harbor City, CA, USA).

Sample collection

A total 33 plum wines were included in this study. Twenty-four plum wines were produced in house, and nine were produced by manufacturer of alcoholic beverages. Of 24 in-house manufactured plum wines, 20 samples (sample 1–20) were manufactured in house at Korea Consumer Agency, and four were produced in-house in small manufacturing facilities. All were obtained by Korea Consumer Agency around March 2011.

Analysis of ethyl carbamate

The ethyl carbamate analytical methods were slightly modified from the Korean Food Code from MFDA [6]. Briefly, 5.0 g of plum wine coupled with 1 mL of 100 µg/mL of butyl carbamate was diluted with 34 mL of distilled water. The mixture was poured into the diatomite solid-phase extraction column (Chem Elut column) and fixed for 4 min. Ethyl carbamate was eluted with 150 mL of dichloromethane into a round-bottom flask. The solvent was evaporated to 1 mL using a rotary evaporator (RE 52, Yamato) at 30 °C. The concentrated residue was rinsed

Table 1 Maximum allowable levels of ethyl carbamate in alcoholic beverages set by different countries

Country	Ethyl carbamate concentration ppb (µg/L) in alcoholic beverages				
	Wine	Fortified wine ^a	Distilled spirits	Sake	Fruit brandy ^b
Canada	30	100	150	200	400
USA	15	60			
Czech Republic	30	100	150	200	400
France			150		1000
Germany					800

^aFruity wines and liqueurs

^bFruity distillates and fruity, mixed and other spirits

with 2 mL of dichloromethane and then transferred to the concentrator tube. The concentrated eluate was made up to an exact 1 mL volume, dried under a stream of nitrogen. The gas chromatograph (CP-3800) equipped with a DB-WAX column (0.25 mm ID, 30 m long, 0.25 μ m film thickness, Agilent, Palo Alto, CA, USA) with a SQ-300 mass spectrometry detector (Bruker, USA) was utilized for the analysis of ethyl carbamate. The specific analysis condition can be found in Table 2. Helium was used as the carrier gas at a constant flow rate of 0.9 ml/min. The oven temperature program was set as follows: 40 °C for 0.75 min, increased to 60 °C at a rate of 10 °C/min, then increased to 150 °C at a rate of 3 °C/min and held at this temperature for 5 min. Ions were obtained by electron impact ionization at 70 eV. The temperature for the injection port was set 180 °C. Splitless injection was used and 1 μ L of extract was injected. The mass spectrometer was operated at an ion source temperature of 200 °C in ionization mode and electron impact at energy of 70 eV. Selected ion-monitoring (SIM: m/z 62, 74, and 89) was used for the analysis of both EC and BC. Ethyl carbamate was identified based on its retention times, and the ion intensity ratio of characteristic mass fragment (m/z) in Table 2 was within $\pm 20\%$ with respect to the theoretical ratio.

Method validation

The different concentrations of 50, 100, 200, 400, 800, 1600 μ g/kg ethyl carbamate (coupled with 100 μ g/ml of butyl carbamate) were injected into GC/MS to make a calibration curve. All samples were prepared in triplicate. To test the recovery of ethyl carbamate, standard solution at levels of 100 μ g/kg were added to sample that did not contain ethyl carbamate. For determining the limit of quantification (LOQ), 50 μ g/kg of ethyl carbamate was injected ten times and the standard deviation was

calculated. The limit of detection (LOD) and LOQ were calculated based on following equations [20]. $LOD = 3 \times SD/Slope$, $LOQ = 10 \times SD/Slope$. The LOD and LOQ of ethyl carbamate were 1.6 and 3.5 μ g/kg, respectively.

Results and discussion

Analytical validation

The standard calibration equations, recovery rates, LOD (limit of detection) and LOQ (limit of quantification) of tested ethyl carbamate can be found in Table 3. The coefficient (R^2) of standard curves was 0.9999 which showed a superior linearity. The recovery rates ranged from 88.1 to 89.1%, the relative standard deviation (RSD) was 3.24%. The LOD and LOQ of ethyl carbamate were 1.6 and 3.5 μ g/kg, respectively.

Ethyl carbamate levels in plum wines

The levels of ethyl carbamate in 33 plum wines can be found in Table 4. Twenty-four plum wines produced in-house showed ethyl carbamate levels from not detectable to 352.7 μ g/kg, with the mean value of 66.5 μ g/kg. The plum wines that had relatively higher level of ethyl carbamate included sample 3 and 9, with 200.7 and 352.7 μ g/kg, and they were produced in year of 2003 and 2007. Nine plum wines produced by manufacturers of alcoholic beverages showed ethyl carbamate levels from not detectable to 295.3 μ g/kg, with the mean of 141.1 μ g/kg. Among commercially produced plum wines (sample 25–33), samples 25 and 26 were produced by sample company, and samples 29–31 were produced by sample company. The samples 29–31 had relatively higher concentrations of ethyl carbamate than other samples with 137.2, 295.3, and 246.8 μ g/kg, and it may have been attributed from the plum extract

Table 2 Analytical conditions for analysis of ethyl carbamate using GC/MS

Parameter	Analytical condition
Column	DB-WAX column (30 m \times 0.25 mm, I.D. 0.25 μ m film thickness)
Column temperature	0.75 min at 40 °C, increase to 60 °C at 10 °C/min, and increase to 150 °C at 3 °C/min and hold for 5 min
Mass spectrometer	Ion source 200 °C Electron impact ionization potential 70 eV Select ion mode(m/z; 62, 74, 89)
Mobile phase	He gas
Flow rate	0.9 mL/min
Injection volume	1 μ L
Injection mode	Splitless

Table 3 Parameters of calibration curves, recovery rates, limit of detection (LOD), and limit of quantification (LOQ) of ethyl carbamate

Standard calibration equation ($y = ax + b$)			Recovery rates (%)	LOD ($\mu\text{g}/\text{kg}$)	LOQ ($\mu\text{g}/\text{kg}$)
a	b	R^2			
204.46	4721.6	0.9999	88.1 ~ 89.1%	1.6	3.5

Table 4 The level of ethyl carbamate in the alcoholic beverages analyzed by GC/MS

No.	Sample description	Year of manufacture	Pit removal	Fruit removal	Fruit removal time	Storage	Sugar	Ethyl carbamate ($\mu\text{g}/\text{kg}$)
1	In-house manufacture	2010	X	O	3 mo	Outdoor	O	6.9 ± 0.65
2		2008	X	X	X	Outdoor	O	44.8 ± 0.09
3		2003	X	X	6 years	Outdoor	O	200.7 ± 3.88
4		2010	X	X	X	Indoor	X	34.7 ± 0.59
5		2008	X	X	X	Outdoor	O	104.1 ± 1.06
6		2008	X	O	3 months	Refrigeration	O	11.3 ± 0.86
7		2008	X	O	1 year	Outdoor	O	N.D. ^a
8		2009	X	O	3 months	Outdoor	X	43.9 ± 0.07
9		2007	X	O	6 months	Outdoor	X	352.7 ± 2.27
10		2009	X	O	3 months	Outdoor	O	15.4 ± 0.02
11		2008	X	O	3 months	Outdoor	O	58.7 ± 1.60
12		2007	X	O	3 months	Outdoor	O	37.7 ± 0.24
13		2009	X	O	6 months	Outdoor	X	52.0 ± 0.93
14		2010	X	X	X	Outdoor	X	17.9 ± 0.11
15		2010	X	O	3 months	Outdoor	O	52.8 ± 0.15
16		2008	X	O	1 year	Indoor	O	143.1 ± 0.35
17		2009	X	O	6 months	Outdoor	O	77.5 ± 1.93
18		2010	X	O	6 months	Outdoor	O	7.2 ± 0.004
19		2010	O	O	3 months	Outdoor	O	12.9 ± 0.008
20		2010	X	X	X	Outdoor	X	9.9 ± 0.11
21	In-house production by small manufacturer	Information not available						40.8 ± 0.29
22								17.8 ± 0.42
23								89.4 ± 0.56
24								97.5 ± 0.14
25	Commercially produced	2014	Plum extract 62%, Wine 38%, Antioxidant, Anhydrous sulfuric acid less than 0.035%					63.8 ± 0.39
26		2014	Plum extract 62%, Wine 38%, Antioxidant, Anhydrous sulfuric acid less than 0.035%					86.4 ± 1.03
27		2014	Plum extract 50%, Wine 42.2%, Spirit, Antioxidant, Anhydrous sulfuric acid less than 0.035%					71.7 ± 0.43
28		2013	Plum extract 80%, Fruit-based spirit 20%					64.3 ± 0.02
29		2014	Plum extract 80%, Fruit-based spirit 20%, Antioxidant, Anhydrous sulfuric acid less than					137.2 ± 0.57
30		2014	Plum extract 80%, Fruit-based spirit 20%, Antioxidant, Anhydrous sulfuric acid less than					295.3 ± 1.38
31		2016	Plum extract 61%, Fruit-based spirit 39%, Antioxidant, Anhydrous sulfuric acid less than					246.8 ± 2.02
32		2015	Plum 45.2%, Alcohol 15%, Sugar 17.5%, Distilled water					163.5 ± 0.03
33		2016	Plum juice 58%, Distilled water, Spirit, fructose					N.D. ^a

^aN.D. not detected

that were included in the plum wine manufacturing. Sample No 32 were imported plum wine from Japan, and had ethyl carbamate level of 163.5 $\mu\text{g}/\text{kg}$, which is higher than average. Sample 33 utilized plum juice for plum wine, in that they simply blend plum juice and spirit with sugar (fructose). Therefore, ethyl carbamate was not detected because no fermentation process was involved. There was no significant difference between homemade plum wine and plum wine produced by a manufacturer of alcoholic beverages. (F-test, $P > 0.05$). Previous study [21] on the levels of ethyl carbamate in brandies derived from stone-fruits, reported the highest level of 100–20,000 $\mu\text{g}/\text{kg}$ found in brandies. The levels of ethyl carbamate in plum wine from this study was higher than the levels from stone fruit brandies reported from their study [21].

Ethyl carbamate is formed in distilled spirit, especially, in stone-fruit spirit (cherries, apricots or plums), via the reaction of ethanol and isocyanate, a byproduct from the enzymatic hydrolysis of cyanoglycosides presenting in the stones of fruits [22]. Hwang et al. [17] also reported that ethyl carbamate of plum wine is formed when a chemical substance (cyanide, cyanate) from the stones reacts with ethanol. The CFS (Centre for Food Safety, the Government of the Hong Kong Special Administrative Region) published the Guidelines to reduce the level of ethyl carbamate in alcoholic beverages during storage and transport [22]. In their report, light exposure and elevated storage temperature are two key factors that influence the formation of ethyl carbamate in wine and distilled spirit during storage and transport. It is known that the formation of ethyl carbamate continues in wine during storage. The rate of formation is accelerated with an increase of the temperature. A significantly increase in formation rate of ethyl carbamate in grape wine has been observed at the temperatures above 38 °C [22]. In this study, the sample 5, 6, and 16 were in-house produced plum wine, manufactured in 2008. When comparing the levels of ethyl carbamate in these three samples, the sample 6 had significantly lower ethyl carbamate than other two samples with 11.3 $\mu\text{g}/\text{kg}$, while sample 5 had 104.1 $\mu\text{g}/\text{kg}$, and sample 16 had 143.1 $\mu\text{g}/\text{kg}$ of ethyl carbamate. The difference among these samples were on storage condition in that the sample 6 were stored in the refrigerator during the fermentation period, therefore the temperature during fermentation was significantly lower than other samples. This result supports the previous finding that the formation rate of ethyl carbamate in alcoholic beverage is influenced by the storage temperature.

Other factors influencing the formation of ethyl carbamate in alcoholic beverage is the degree of fermentation. Hwang et al. [17] reported the increase of ethyl carbamate in the plum wines with 16 or 30% alcohol content, during the fermentation period. The concentrations of ethyl carbamate in plum wines with alcohol content of 15–30%,

reached its highest with 0.071 and 0.188 $\mu\text{g}/\text{g}$ after 6 months fermentation. The Technical Service Institute of National Tax Service of Korea (2007) conducted a survey on the quality improvement of alcoholic drinks, and found the linear relationship between alcoholic content and level of ethyl carbamate [23]. Ethyl carbamate was detected as 97.28 $\mu\text{g}/\text{kg}$ when the alcohol content was 40% and 149.03 $\mu\text{g}/\text{kg}$ when the alcohol content was 55%. In this study, the levels of ethyl carbamate was higher in sample 31 with 246.8 $\mu\text{g}/\text{kg}$, than the levels found in sample 29 with 137.2 $\mu\text{g}/\text{kg}$. When looking at the fruit-based spirit in two plum wines, sample 29 contained about 20% fruit-based spirit, while sample 31 had 39% of fruit-based spirit. The levels of alcoholic content was higher in sample 31, so as ethyl carbamate. This study also supports the previous reports on higher formation of ethyl carbamate in alcoholic beverages with higher alcoholic content.

In addition, the longer the leach period of plum wine, the higher the production of ethyl carbamate. Ethyl carbamate was 7.76 $\mu\text{g}/\text{kg}$ in the first week, 42.14 $\mu\text{g}/\text{kg}$ in the sixth week and 97.28 $\mu\text{g}/\text{kg}$ in the 12th week when the alcohol content was 40%. When the alcohol content was 55%, ethyl carbamate was 20.26 $\mu\text{g}/\text{kg}$ in the first week, 67.79 $\mu\text{g}/\text{kg}$ in the sixth week, and 149.03 $\mu\text{g}/\text{kg}$ in the 12th week. When leached with the same plums, the amount of ethyl carbamate increased with an increase of the leaching temperature. At 170 kg of plum and alcohol content of 50%, it was detected as 113.9 $\mu\text{g}/\text{kg}$ at 20 °C and 247.5 $\mu\text{g}/\text{kg}$ at 35 °C. Based on these studies, the Korean Ministry of Food and Drug Safety published the Manual for Ethyl Carbamate of Alcoholic Beverages Reduction [24] and, according to this manual, the alcohol content of plum wine should be reduced to below 50% of ethanol, plum wine should be stored at room temperature and the dipping period of plum should not exceed 100 days.

In this study, the concentration of ethyl carbamate in plum wines (produced both in house and by manufacturers of alcoholic beverages) was analyzed by GC–MS. The maximum level of ethyl carbamate in plum wine manufactured at home was 352.7 $\mu\text{g}/\text{kg}$. Therefore, the government needs to inform the public of the Manual for Ethyl Carbamate of Alcoholic Beverages Reduction and regularly monitor the content of ethyl carbamate in plum wine. In addition, the standard for ethyl carbamate in plum wine needs to be considered.

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References

1. Chu MY. China Fruit Flora. Japanese Apricot. China Forest Press. Beijing, China. (1999)
2. Shen YY, Ding XJ, Wang F, Cai BH, Gao ZH, Zhang Z. Analysis of genetic diversity in Japanese apricot (*Prunus mume* Sieb. et Zucc.) based on REMAP and IRAP molecular markers. *Scientia Horticulturae* 132, 50–58 (2011)
3. Chuda Y, Ono H, Ohnishi-Kameyama M, Matsumoto K, Nagata T, Kikuchi YM. Citric acid derivative improving blood fluidity from fruit-juice concentrate of Japanese apricot (*Prunus mume* Sieb. et Zucc.). *J. Agric. Food Chem.* 47, 828–831 (1999)
4. Jeong JT, Moon JH, Park KH, Shin CS. Isolation and characterization of a new compound from *Prunus mume* fruit that inhibit cancer cells. *J. Agric. Food Chem.* 54, 2123–2128 (2006)
5. Ryu D, Koh E. Determination of ethyl carbamate in commercial and homemade Maesilju. *Journal of East Asian Society of Dietary Life.* 25(2), 309–315 (2015)
6. Korean Ministry of Food and Drug Safety(MFDA). Reduction Manual of ethyl carbamate in alcoholic beverages. Republic of Korea. (2011)
7. Lachenmeier DW, Kanteres F, Kuballa T, Lopez MG, Rehm J. Ethyl carbamate in alcoholic beverages from Mexico (Tequila, Mezcal, Bacanora, Sotol) and Guatemala (Cuxa): Market survey and risk assessment. *Int. J. Environ. Res. Public Health.* 6, 349–360 (2009)
8. Schehl B, Senn T, Lachenmeier DW, Rodicio R, Heinisch JJ. Contribution of the fermenting yeast strain to ethyl carbamate generation in stone fruit spirits. *Appl. Microbiol. Biotechnol.* 74, 843–850 (2007)
9. Weber JV, Sharypov VI. Ethyl carbamate in foods and beverages: A review. *Environ. Chem. Letters.* 7, 233–247 (2009)
10. Beland FA, Benson RW, Millick PW, Kovatch RM, Robert DW, Fang JL, Doerge DR. Effect of ethanol on the tumorigenicity of urethane (ethyl carbamate) in B6C3F1 mice. *Food Chem. Toxicol.* 43, 1–19 (2005)
11. IARC. IARC monographs on the evaluation of carcinogenic risks to humans. Available from: <http://monographs.iarc.fr/ENG/Monographs/vol96/mono96.pdf> (2010).
12. Melo Abreu S, Alves A, Oliveira B, Herbert P. Determination of ethyl carbamate in alcoholic beverages: An interlaboratory study to compare HPLCFLD with GC–MS methods. *Analytical Biochem.* 382, 498–503 (2005)
13. Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO). Prepared by the sixty-fourth meeting of the joint FAO/WHO expert committee on food additives (JECFA) FAO. *Food Nutr.* 82:1–778 (2006)
14. Hasnip S, Crews C, Potter N, Christy J, Chan D, Bondu T, Matthews W, Walters B, Patel K. Survey of ethyl carbamate in fermented foods sold in the United Kingdom in 2004. *J. Agric. Food Chem.* 55:2755–2759 (2007)
15. Foulk JE. Urethane in alcoholic beverages under investigation. U. S. FDA/CFSAN Consumer Report. Washington, USA. (1993)
16. Horii S, Goto K. Determination of ethyl carbamate in Sake using headspace solid phase microextraction. *J. Inst. Brew.* 116:177–181 (2010)
17. Hwang LH, Kim AK, Park KA, Kim JY, Hwang IS, Chae YZ. The Effect of Raw Material, Alcohol Content, and trans-Resveratrol on the Formation of Ethyl Carbamate in Plum Wine. *J. Fd Hyg. Safety.* Vol. 24, No.3, pp. 194–199 (2009)
18. Kim YK, Koh EM, Chung HJ, Kwon HJ. Determination of ethyl carbamate in some fermented Korean foods and beverages. *Food Add Contam.* 17:469–475 (2000)
19. Hong KP, Roh IW, Kang YS, Jung DC, Park SR, Yoon JH, Bae DH. Monitoring and Risk Assessment of Ethyl Carbamate in Korean Major Foods. *J. Korean Soc. Appl. Biol. Chem.* 50(1), 29–35 (2007)
20. Harris DC. Exploring chemical analysis. W.H. Freeman and Company, New York, NY p 91–98. (2001)
21. Battaglia R, Conacher HBS, Page BD. Ethyl carbamate(urethane) in alcoholic beverages and food. *Food Addit. Contamin.* 4, 477–496 (1990)
22. The Government of the Hong Kong Special Administrative Region, Centre for Food Safety. Guidelines to the trade on reducing the level of ethyl carbamate(EC) in alcoholic beverages during storage and transport. Hong Kong, China. (2009)
23. Technical Service Institute of National Tax Service of Korea. Research on quality improvement of alcoholic drinks. Republic of Korea. (2007)
24. Korean Ministry of Food and Drug Safety (MFDA). Monitoring and risk assessment of ethyl carbamate in alcoholic beverages. Republic of Korea. (2004)