Food Additives and Contaminants, May 2005; 22(5): 397–405

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Retrospective trends and current status of ethyl carbamate in German stone-fruit spirits

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(Received 25 November 2005; revised 8 February 2005; accepted 8 February 2005)

Abstract
Ethyl carbamate (urethane, $C_2H_5OCONH_2$) is a known genotoxic carcinogen of widespread occurrence in fermented food and beverages with highest concentrations found in stone-fruit spirits. Between 1986 and 2004, 631 cherry, plum or mirabelle (yellow plum) spirits were analysed for ethyl carbamate using gas chromatography in combination with mass spectrometry after extrelut extraction. The ethyl carbamate concentration of the samples ranged between 0.01 mg l^{-1} and 18 mg l^{-1} (mean 1.4 mg l^{-1}). After exposure of the samples to UV light, significantly ($p = 0.001$) higher concentrations between 0.01 mg l^{-1} and 26 mg l^{-1} (mean 2.3 mg l^{-1}) were found. The ethyl carbamate concentration increased on average by 1.3 mg l^{-1}. A linear correlation between the year of sampling and ethyl carbamate concentration showed a statistically significant but very slight decrease ($R = -0.10$, $p = 0.024$). However, if only samples which officially were non-compliant were considered exceeding the upper limit of 0.4 mg l^{-1} more than twice, a significant reduction ($R = -0.56$, $p = 0.018$) of the quota was evident. This shows that measures to reduce ethyl carbamate were successfully introduced in many distilleries. However, nearly 20 years after the first warnings about ethyl carbamate in spirit drinks, the problem persists especially in products derived from small distilleries. During experimental production of stone-fruit spirits using state-of-the-art technologies, it was shown that the occurrence of ethyl carbamate in stone fruit spirits is preventable. Even for small distilleries, simple possibilities like destoning exist to minimize the ethyl carbamate content.

Keywords: *Ethyl carbamate, hydrocyanic acid, stone fruit spirits, cherry spirit, plum spirit, mirabelle spirit,* Prunus *L*

Introduction

Ethyl carbamate (urethane, $C_2H_5OCONH_2$) is a known genotoxic carcinogen of widespread occurrence in fermented food and beverages (Dennis et al. 1989; Battaglia et al. 1990; Schlatter and Lutz 1990; Zimmerli and Schlatter 1991; Sen et al. 1992; Sen et al. 1993; Benson and Beland 1997; Kim et al. 2000). Public health concern of ethyl carbamate in alcoholic beverages began in 1985 when relatively high levels were detected by Canadian authorities including spirit drinks imported from Germany (Conacher and Page 1986). The highest ethyl carbamate concentrations were found in spirits derived from stone fruit of the species *Prunus* L. (Rosaceae) (like cherries, plums, mirabelles (yellow plums), or apricots) (Battaglia et al. 1990; Zimmerli and Schlatter 1991). Subsequently, Canada established an upper limit of 0.4 mg l^{-1} ethyl carbamate for fruit spirits (Conacher and Page 1986), which was adopted by Germany and many other countries.

The formation of cyanogenic glycosides such as amygdalin in stone fruit by enzymatic action (mainly β-glucosidase) leads to the generation of cyanide, which is the most important precursor of ethyl carbamate in spirits. Cyanide is oxidized to cyanate, which reacts with ethanol to form ethyl carbamate (Wucherpfennig et al. 1987; Battaglia et al. 1990; MacKenzie et al. 1990; Taki et al. 1992; Aresta et al. 2001). The wide range of ethyl carbamate concentrations in stone-fruit spirits reflects its light-induced and time-dependent formation after distillation and storage (Andrey 1987; Mildau et al. 1987; Baumann and Zimmerli 1988; Zimmerli and Schlatter 1991; Suzuki et al. 2001).

Many preventive actions to avoid ethyl carbamate formation in alcoholic beverages have been

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ISSN 0265–203X print/ISSN 1464–5122 online
DOI: 10.1080/02652030500073360

proposed. Besides, self-evident measures of good manufacturing practice like the use of high-quality, non-spoiled raw material, and high standards of hygiene during fermentation and storage of the fruit mashes (Dürr 1992; Lafuente and Fabre 2000), the mashing and distillation conditions must be optimized. To avoid the release of cyanide, it is essential to avoid breaking the stones, to minimize light irradiation, and to shorten storage time (Christoph and Bauer-Christoph 1998). Some authors have proposed the addition of enzymes to decompose cyanide or a complete destoning of the fruit prior to mashing. The mashes have to be distilled slowly with an early switch (at 65% (v/v)) to the tailing-fraction (Dürr 1992). Further preventive actions are the addition of patented copper salts to precipitate cyanide in the mash (Christoph and Bauer-Christoph 1998; Christoph and Bauer-Christoph 1999), the distillation using copper catalysts (Pieper et al. 1992a; Kaufmann et al. 1993) or the application of steam washers (Nusser et al. 2001). However, the use of copper can generate environmental problems due to hazardous waste.

Materials and methods

Sample collective

Between 1986 and 2004, 631 stone-fruit spirits submitted to the CVUA Karlsruhe were analysed for ethyl carbamate. Our institute covers as a part in official food control in Baden-Württemberg the district of Karlsruhe in North Baden (Germany), which has a population of approximately 2.7 million and includes the northern part of the Black Forest, a territory with around 14 000 approved distilleries (including South Baden) producing well-known specialties like Black Forest Kirsch (cherry spirit). The sampling was conducted by local authorities directly at the distilleries or from retail trade. Generally, spirits already diluted to drinking strength as offered to the end-consumer were taken. Since 2001, an interview protocol at sampling has been made including questions about preventive actions, age of the distillery, cleaning of the distillery, fermentation conditions, storage of the fruit mashes, and distillation conditions in general. To eliminate the possibility of ethyl carbamate formation during transport and sample storage, the bottles were wrapped in aluminium foil directly after sampling.

Experimental production of stone-fruit spirits

To show the state-of-the-art in the production of stone-fruit spirits in comparison to commercial samples, cherry and plum spirits of different vintages were produced under completely standardized conditions at the Institute of Fermentation Technology Hohenheim. Thereby appropriate and commonly employed commercial available yeast strains were used. All strains were purchased from Begerow GmbH & Co. (Langenlonsheim, Germany). Media, culture conditions and incubation of the yeast strains were standardized and carried out according to Schehl et al. (2004).

Raw material and mashing process

The studies were performed with two different stone-fruit mashes: cherries (cv. *Dollenseppler*) and plums (cv. *Ersinger Frühzwetschge*). The cherries were in an excellent condition like fresh dessert fruit, no bruised or decayed fruit were present. The plums were in faultless but in a bit more critical condition, so that single foul fruit were sorted out.

Mashes were prepared according to standard procedures. Indeed the fruit (exempted from peduncles) were washed and chopped using a stirrer attached to a drill machine, so that the stones remained undamaged (see Hagmann 2002) and then divided into equal lots. One fraction was not treated any further (further named as *complete mashes*), the other portion was passed through a pulping machine and destoner (filter-width 4 mm, capacity 50–250 $kg\,h^{-1}$; Bockmeyer, Nürtingen, Germany) for the total removal of the stones (further named as *stoneless mashes*). Immediately after comminution respectively pitting the fruit, the pH-value was adjusted to 3.0 with sulphuric acid (technical grade). The remaining stones were collected and fermented separately without addition of sulphuric acid.

Fermentation

The mash was divided in 90 kg-lots each and separated in 120 l vessels. For fermentation, the vessels were sealed with a fermentation bung and inoculated with the selected yeast strains (all standardized to be in the same physiological state and cell density) and fermented to completion at 15–17°C. All experiments were performed in triplicate and the classical fermentation parameters were observed over the whole fermentation period (for details see Schehl et al. 2004). The remaining stones were separately fermented and distilled.

Distillation

The distillation was accomplished under technical and standardized conditions using a 200 l copper pot still (Jacob-Carl, Göppingen, Germany) fitted with an enrichment section consisting of three bubble plates, a dephlegmator and a copper catalyst (Holstein, Markdorf, Germany). The dephlegmator was run

with a flow rate of 120 lh^{-1} and the copper catalyst was used. The fermented mashes were distilled with two plates in operation. The distillates were collected in fractions with a volume of 250–300 ml, each. In the vicinity of the switching points (heads to product fractions and product fractions to tailings) smaller volumes of 150 ml were collected. The heads were identified with the detaching test determining acetaldehyde according to Pieper et al. (1987). The tailings were screened by detachment at 72% (v/v) and partly by organoleptic assessment. The stones were distilled on a 191 plant with three plates, a dephlegmator and without a catalyst. Fractions were collected and the heads and tailings discarded.

Spirit fractions

The product fractions were stored for at least one week at 17°C, then diluted with deionized water to an alcohol content of 40% (v/v), cold filtered at 4°C (Macherey Nagel, Düren, Germany) and stored in darkness for another four weeks at 17°C prior to further analysis.

Quantitative determination of ethyl carbamate and cyanide

The analysis of ethyl carbamate was done using previously published procedures combining the extrelut extraction procedure of Baumann and Zimmerli (1986) with gas chromatography and mass spectrometry (GC/MS) according to Mildau et al. (1987) (analyses 1986–2003) or tandem mass spectrometry (GC/MS/MS) according to Lachenmeier et al. (2004) (analyses in 2004). For sample preparation, 20 ml of stone-fruit spirit or 20 ml of filtrated mash were spiked with 50 µL of ethyl carbamate-d_5 (1 mg ml^{-1}) that was synthesized according to Funch and Lisbjerg (1988), and directly applied to the extraction column. The extrelut column was wrapped in aluminium foil to eliminate the possibility of ethyl carbamate formation during extraction. After 15 min of equilibration, the column was washed with 2×2 ml of n-pentane. Next, the analytes were extracted using 3×30 ml of dichloromethane. The eluates were combined in a brown flask and reduced to 2–3 ml in a rotary evaporator (30°C, 300 mbar). After that, the solution was adjusted to 10 ml with ethanol in a measuring flask and directly injected into the GC/MS or GC/MS/MS system. In addition, to evaluate the light-induced ethyl carbamate formation capability of the products, the samples were exposed to UV light for 4 hr using a 360 W high-pressure mercury lamp Psorilux 3060 (Heraeus, Hanau, Germany) and extracted as described above. The recovery of ethyl carbamate was $100.4 \pm 9.4\%$. The limit of detection was 0.01 mg l^{-1} of ethyl carbamate. The precision never exceeded 7.8% (intraday) and 10.1% (interday) as well as the trueness never exceeded 11.3% (intraday) and 12.2% (interday), indicating good assay accuracy (Lachenmeier et al. 2004).

The total hydrocyanic acid (HCN) in the stone-fruit spirits was photometrically determined after hydrolysis with potassium hydroxide and reaction with chloramine-T and pyridine/barbituric acid reagent using the method of Wurzinger and Bandion (1985). For the determination of mashes, hydrocyanic acid was separated from the matrix by distillation before the photometric analysis (Wurzinger and Bandion 1993). The limit of detection was 0.15 mg l^{-1} of hydrocyanic acid.

Statistics

All data were evaluated using standard statistical packages for Windows. Statistical significance was assumed at below the 0.05 probability level. Groups of two cases were compared using *t*-tests. One-way analysis of variance (ANOVA) was used to test whether three or more cases have the same mean including the Bonferroni post hoc means comparison. Pearson's test was used to evaluate the significance of linear relations.

Results

The results of 631 analysed stone-fruit spirit samples from commercial trade are given in Table I. The ethyl carbamate concentration of the samples ranged between 0.01 mg l^{-1} and 18 mg l^{-1} (mean 1.4 mg l^{-1}). After exposing of the samples to

Table I. Ethyl carbamate concentrations of 631 stone-fruit spirits. The samples were collected and measured over a period of 19 years.

	All samples (Total)		Cherry		Plum		Mirabelle	
	OS	UV	OS	UV	OS	UV	OS	UV
N	631	538	312	256	212	187	107	95
Positive	89%	88%	93%	93%	83%	81%	86%	87%
Mean ± SD (mg l^{-1})	1.4 ± 1.7	2.3 ± 3.2	1.5 ± 1.9	2.7 ± 3.5	1.2 ± 1.5	1.8 ± 2.6	1.2 ± 1.6	2.3 ± 3.0
Range (mg l^{-1})	0.01–18	0.01–26	0.01–18	0.06–26	0.01–8.8	0.01–16.5	0.06–9.2	0.07–11.8
Median (mg l^{-1})	0.74	1.05	1.0	1.5	0.6	0.5	0.6	0.8

OS: original samples; UV: 4 h irradiated samples; SD: standard deviation.

Table II. Light-induced formation of ethyl carbamate after exposition to UV light (4 h).

	All samples (Total)	Cherry	Plum	Mirabelle
N	538	256	187	95
Samples with formation	69%	77%	55%	72%
Increase mean ± SD ($mg\,l^{-1}$)	1.3 ± 2.4	1.5 ± 2.7	1.0 ± 1.7	1.4 ± 2.2
Increase range ($mg\,l^{-1}$)	0.01–21	0.01–21	0.01–11	0.01–9
Increase median ($mg\,l^{-1}$)	0.4	0.5	0.3	0.4

UV light, significantly ($p = 0.001$) higher concentrations between 0.01 $mg\,l^{-1}$ and 26 $mg\,l^{-1}$ (mean 2.3 $mg\,l^{-1}$) were determined. Using ANOVA, no significant difference between the three fruit groups in the ethyl carbamate content could be determined for the dark-stored samples ($p = 0.07$). However, after irradiation with UV light, a significant difference of the mean could be proven between cherry and plum spirit, but not between the cherry and mirabelle or plum and mirabelle (ANOVA $p = 0.03$). The ethyl carbamate concentration increased in average by 1.3 $mg\,l^{-1}$ (see Table II), with the highest formation capability usually found in cherry spirits. However, on average the formation capability of all fruit groups is the same (ANOVA $p = 0.20$). Figure 1 and Table III show the distribution of the ethyl carbamate concentrations between different concentration categories. More than 50% of the samples had ethyl carbamate concentrations above the Canadian upper limit.

Figure 2 visualizes the retrospective trend of ethyl carbamate in German stone-fruit spirits analysed since 1986. Using ANOVA, a significant difference between the means could be determined ($p = 0.002$). However in the post hoc means comparison, there were no significant differences between any of the sub groups. Therefore, no consistent trend could be seen. If a linear correlation is done between the year of sampling and the ethyl carbamate concentration, a statistically significant but only very slight decrease ($R = -0.10$) was found (see Table IV). All in all, our data state that the average ethyl carbamate content of stone-fruit spirits remains nearly constant over the years. However, if only officially complained samples are considered exceeding the upper limit of 0.4 $mg\,l^{-1}$ more than twice, a significant reduction of the quota could be proven (Figure 3). In 1986, more than 65% of the analysed samples had to be rejected. Nowadays, the rejection quota varies between 25% and 40%.

The HCN concentration of the samples ranged between 0.15 and 22 $mg\,l^{-1}$ (mean 1.96 ± 2.52 mg l^{-1}). No correlation could be found between ethyl carbamate and its main precursor cyanide, neither for the dark-stored samples nor for the UV-irradiated samples (Table IV). There

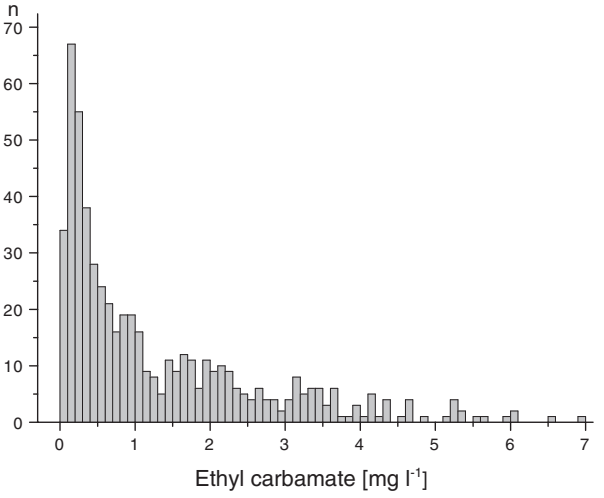

Figure 1. Statistical distribution of ethyl carbamate concentrations in 631 stone-fruit spirits analysed between 1986 and 2004.

Table III. Distribution of ethyl carbamate concentrations.

	All samples		Cherry		Plum		Mirabelle	
	OS	UV	OS	UV	OS	UV	OS	UV
N	631	538	312	256	212	187	107	95
Nd	11%	12%	7%	7%	17%	18%	14%	13%
<0.4 $mg\,l^{-1}$	31%	27%	29%	26%	32%	34%	32%	19%
0.4-0.8 $mg\,l^{-1}$	14%	13%	13%	11%	13%	9%	21%	24%
>0.8 $mg\,l^{-1}$	44%	48%	51%	56%	38%	39%	33%	44%

OS: original samples; UV: 4 h irradiated samples; nd: not detected.

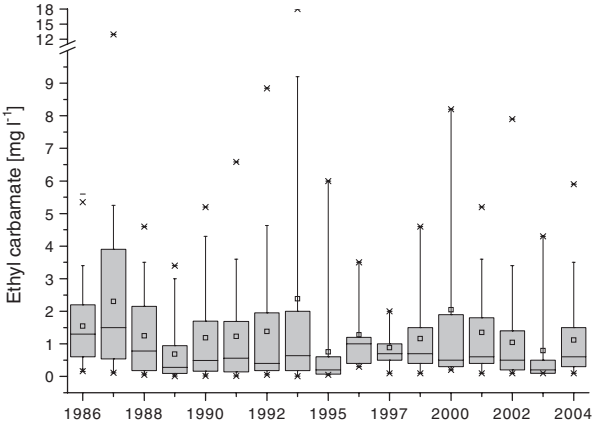

Figure 2. Box-plots for the ethyl carbamate concentrations in 631 stone-fruit spirits analysed between 1986 and 2004 (no data was available for 1994 and 1998). Only a minor reduction ($R = -0.096$) could be proven over this period of time.

Table IV. Results of linear correlation between ethyl carbamate concentrations of original or UV irradiated samples and year of sampling (1986–2004), concentration of total hydrocyanic acid (HCN) as well as the age of the used distillery.

Correlation of Ethyl carbamate with	N	Original sample R	Original sample P	UV irradiated sample R	UV irradiated sample p
Year of sampling	559	−0.096	0.024	−0.146	0.001
HCN	132	0.118	0.180	0.141	0.107
Age of distillery	39	−0.259	0.116	−0.418	0.008

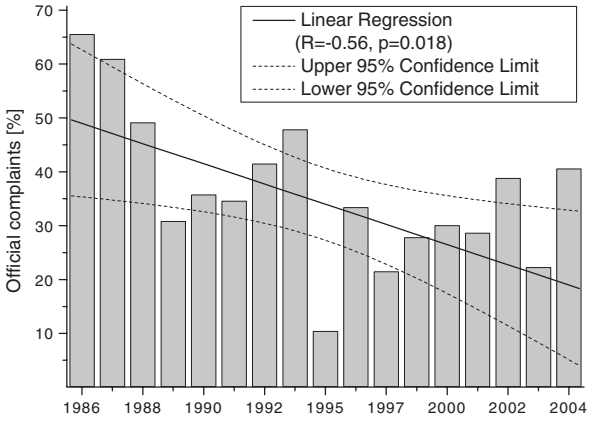

Figure 3. Percentage of samples with ethyl carbamate concentrations higher than $0.8\,mg\,l^{-1}$, which lead to official complaints. A significant reduction ($R=-0.56$) of the quota could be proven between 1986 and 2004.

Table V. Ethyl carbamate concentrations of hydrocyanic acid (HCN) negative and positive cases.

	n	Ethyl carbamate ($mg\,l^{-1}$) Original sample	Ethyl carbamate ($mg\,l^{-1}$) UV irradiated sample
HCN negative	142	0.42 ± 0.75 (0.01–4.64)	0.48 ± 0.97 (0.01–6.65)
HCN positive	138	1.92 ± 2.40 (0.06–18)	3.61 ± 4.23 (0.07–26)
P		<0.0001	<0.0001

Table VI. Ethyl carbamate concentrations of cases with and without the use of preventive actions to avoid the contaminant.

	n	Ethyl carbamate ($mg\,l^{-1}$) Original sample	Ethyl carbamate ($mg\,l^{-1}$) UV irradiated sample
Copper catalyst	12	0.28 ± 0.29 (0.08–1)	0.32 ± 0.35 (0.07–1.2)
No preventive actions	40	1.32 ± 1.44 (0.06–7.88)	1.86 ± 1.84 (0.09–8.7)
P		0.0079	0.0073

was also no correlation between hydrocyanic acid and the light induced increase of ethyl carbamate ($R=-0.06$, $p=0.51$). However if the ethyl carbamate concentrations of HCN-negative and HCN-positive samples are compared, the positive ones showed a significantly higher ethyl carbamate concentration and, of course, a higher formation capability (Table V).

If the interview protocols are considered, a significant negative correlation was found between the age of distillery and the ethyl carbamate content after irradiation (Table IV), attributed to the fact that new distilleries are usually equipped with copper catalysts or other preventive measures. The comparison between ethyl carbamate concentrations of spirits produced using copper catalysts and spirits produced without preventive actions confirms this relation. The samples distilled over copper catalysts (apart from a single distillate with $1\,mg\,l^{-1}$) had a significantly lower ethyl carbamate concentration below the upper limit (Table VI). No correlation between the other information regarding the interview protocol like mash storage time or state of cleaning of the distillery and ethyl carbamate or hydrocyanic acid content could be made.

The results of the experimental and standardized production of stone-fruit spirits are shown in Tables VII and VIII. Apart from one sample with a very low concentration, ethyl carbamate was not detected in any of the mashes. Hydrocyanic acid was found in concentrations between 0.7 and $4.7\,mg\,l^{-1}$ with lower or not detectable contents in the stoneless mashes than in the complete mashes. In the spirits of the years 2002–2003 (distilled from the complete and stoneless mashes), no ethyl carbamate was detected. In contrast, the stones had a very high concentration of hydrocyanic acid after fermentation, and the ethyl carbamate concentration in the distillate exceeded the upper limit. Two cherry spirits from the year 2004 showed low values of ethyl carbamate ($0.2\,mg\,l^{-1}$ in the complete mash and $0.1\,mg\,l^{-1}$ in the stoneless mash). In these positive samples, the ethyl carbamate concentrations were below the upper limit; only the 'complete mash' sample had the capacity for ethyl carbamate formation up to $1\,mg\,l^{-1}$. Therefore, the results from 2004 show that removing the stones reduced the hydrocyanic acid concentration in the mash and hence the ethyl carbamate content in the distillate as well as the formation capability (based on good technological manufacturing).

Discussion

Food regulatory viewpoints

In our study, an enormously wide range of ethyl carbamate concentrations were found in stone-fruit spirits, varying in more than three orders of

Table VII. Ethyl carbamate (EC) and hydrocyanic acid (HCN) concentrations of standardized produced stone fruit mashes intended to produce spirit drinks. The fruit derived always from the same cultivation and region. The mashes were treated standardized but in different technological ways with and without stones.

Fruit	Mash treatment	Status	EC OS ($mg l^{-1}$)	EC UV ($mg l^{-1}$)	HCN ($mg l^{-1}$)
Vintage 2003					
Cherry	Complete	Unfermented	nd	nd	0.7
		Fermented	0.1	0.1	4.7
Cherry	Stoneless	Unfermented	nd	nd	1.3
		Fermented	nd	nd	1.4
Plum	Complete	Unfermented	nd	nd	nd
		Fermented	nd	nd	1.3
Plum	Stoneless	Unfermented	nd	nd	nd
		Fermented	nd	nd	nd
Vintage 2004					
Cherry	Complete	Unfermented	nd	nd	nd
		Fermented	nd	nd	4.0
Cherry	Stoneless	Unfermented	nd	nd	nd
		Fermented	nd	nd	0.9
Cherry	Stones	Unfermented	nd	nd	nd
		Fermented	nd	nd	18.7

OS: original samples: UV: 4 h irradiated samples; nd: not detected.

Table VIII. Ethyl carbamate (EC) and hydrocyanic acid (HCN) concentrations of spirits produced by state-of-the-art technology with and without stones. The fruit were collected during seasons over the years 2002–2004. The mashes were treated as described in materials and methods. The spirits were produced under controlled and standardized conditions.

Fruit	Mash treatment	EC OS ($mg l^{-1}$)	EC UV ($mg l^{-1}$)	HCN ($mg l^{-1}$)
Vintage 2002				
Cherry	Complete	nd	nd	nd
Plum	Complete	nd	nd	nd
Vintage 2003				
Cherry	Complete	nd	nd	nd
Cherry	Stoneless	nd	nd	nd
Plum	Complete	nd	nd	nd
Plum	Stoneless	nd	nd	nd
Plum	Stones	1.9	4.0	4.8
Vintage 2004				
Cherry	Complete	0.2	1.0	nd
Cherry	Stoneless	0.1	0.3	nd

OS: original samples; UV: 4 h irradiated samples; nd: not detected.

magnitude, which corresponds well to the results of previous studies (Zimmerli and Schlatter 1991; Adam and Postel 1992). The statistical distribution of our samples corresponds also to that of a study of Andrey (1987), who analysed 135 Swiss cherry spirits, resembling a normal distribution. However, our study found more samples with a higher ethyl carbamate content. These samples were officially rejected, because they were produced contrary to European law. According to Council Regulation (EEC) No 315/93 laying down Community procedures for contaminants in food (Council of the European Communities 1993), no food containing a contaminant in an amount unacceptable from the public health viewpoint and in particular at a toxicological level shall be placed on the market. Furthermore, contaminant levels shall be kept as low as reasonably can be achieved by following good practices. In our opinion, an offence against good practices can be assumed, if the upper limit is exceeded more than twice. In consideration of lot-to-lot differences and inhomogeneities, the manufacturers were advised of their duty to exercise diligence and to use the state-of-the-art measures needed to reduce the content of ethyl carbamate.

In 1999, the German health authorities stated that measures taken so far by manufacturers to reduce ethyl carbamate levels have led to a drop in contamination particularly in products from large distilleries (BgVV 1999). In principle, this statement is in full accordance to our results. The decrease in the rejection quota since 1986 impressively documents that the measures were successively introduced in the distilleries. However, as the relatively stable mean ethyl carbamate concentrations document, this process is very slow. And from our

experience, the problem encompasses particularly small distilleries, which have not introduced improved technologies. In this context it must be stated that our sampling was biased towards those small distilleries, which are often one-man businesses. In the context of a risk assessment, the authorities included more of those types of distilleries and products for sampling that were likely to pose a hazard to the consumer. The few large distilleries, producing for the mass market, have all introduced the described good manufacturing practices and produce stone-fruit distillates with only traces of ethyl carbamate.

Light-induced formation as risk for the consumer

In spite of the efforts of official food control to prevent ethyl carbamate formation after sampling, this concentration reflecting the status after bottling or in trade is not really of interest to the consumer. Only the ethyl carbamate concentration at consumption would be relevant. In many cases this is the maximum content because spirit drinks are usually not stored light-protected either in trade or by the consumers. Therefore, to achieve a better consumer protection, the ethyl carbamate formation capability of stone-fruit spirits should be evaluated in food control. As the results show, the ethyl carbamate concentration regularly increased over the upper limit after irradiation with UV light. Regrettably, the results published by our working group in 1987 (Mildau et al. 1987), which showed significant delay of ethyl carbamate formation in brown glass bottles, did not start a process of re-examination of the use of the traditional white glass bottles. The use of UV filters in the white glass nowadays proposed by some breweries to prolong the shelf-life of beer could be a novel alternative to reduce the formation of ethyl carbamate.

Cyanide as precursor of ethyl carbamate

The findings of several authors that besides cyanide one or several further factors are additionally needed to form ethyl carbamate in stone-fruit distillates are confirmed by our results. Besides light, the factors influencing ethyl carbamate formation from cyanide are pH, ethanol content, temperature, vicinity of carbonyl groups in organic molecules and concentration of copper or iron-ions in the beverage (Baumann and Zimmerli 1988; Battaglia et al. 1990; Aresta et al. 2001). But ethyl carbamate is also found in a variety of fermented beverages and foods (Ough 1976). It is proposed that ethyl carbamate derives from different yeast metabolites such as urea (Pretorius 2000). Nevertheless, urea causes only negligible low values of ethyl carbamate in this context; the main influencing factor for the formation of ethyl carbamate is cyanide, deriving from the stones of the fruit.

In contrast to the study of Aresta et al. (2001), who found a relatively high correlation ($R = 0.597$) between cyanide and ethyl carbamate in Brazilian sugar cane spirits, we only found a very low correlation between these parameters. However, as it is shown in Table V, the determination of cyanide can be used as a simple screening for ethyl carbamate. If cyanide is negative, the ethyl carbamate concentration can be assumed to be below the upper limit. This is in accordance to previous research that no ethyl carbamate is formed in appreciable amount under light exposure when the distillates are free of cyanide (Baumann and Zimmerli 1988). The advantage is that simple test-kits for cyanide are available, which can be used directly at the distilleries for product control, whereas ethyl carbamate analysis is only possible in specialized laboratories.

Reduction of ethyl carbamate

Because of its carcinogenic and mutagenic properties, no limit value below which health risks could be reliably excluded can be formulated for ethyl carbamate. Therefore, the goal must be to consistently reduce the contents by means of technological measures (BgVV 1999). The first priority has to be the quality of the raw material and hygiene during fermentation, distillation and storage. The content of cyanide in the mash depends on the condition of the fruit. Damaged and microbiologically spoiled fruit contain more free cyanide (Hesford 1998). This is confirmed by the observation that samples with an ethyl carbamate content above the upper limit often also contain high levels of propanol-1 or butanol-2. These alcoholic congeners indicate an unwanted fermentation by spoilage micro-organisms (Frank 1983). Pieper et al. (1992b) stated that the formation of ethyl carbamate can be avoided by a defined and careful procedure in the production of stone-fruit spirits.

To reduce the ethyl carbamate levels as low as technologically possible, the use of further measures like copper catalysts is advisable, which cause a significant reduction during distillation. However, it should be noted that the catalysts have to be regularly cleaned and maintained (Hesford 1998). Otherwise, ethyl carbamate concentrations above the upper limit are nevertheless possible.

Destoning to eliminate the precursor cyanide

Copper catalysts or other techniques to reduce ethyl carbamate were primarily established by large distilleries, whereas small distilleries could not afford

the investment or had problems with correct maintenance in the daily routine. Therefore, simpler possibilities to avoid ethyl carbamate are required that must be both economical and adaptable by small distilleries. Since the discovery of cyanide as the main ethyl carbamate precursor, the simplest alternative would be to remove the stones prior to mashing, and therefore remove the precursor cyanide, which is bound as glucoside inside of the stones. Such destoned mashes do not have the potential to form ethyl carbamate during distillation, so that no further measures would be required. However, for a long time, this method was restricted because the possibility to distil high-quality spirits from destoned mashes was questioned (Pieper et al. 1992b). The distillates were described as not typical of the fruit (Dürr 1992) or the sensory quality as not satisfactory (Kaufmann et al. 1993). Nowadays, a process of rethinking has begun. Of course, the destoned distillates do not have the typical and often appreciated 'stone flavour', which is induced by the bitter almond aroma of benzaldehyde. However, this has the advantage that the typical flavour of the fruit itself can now clearly emerge. In addition, the consumer can significantly better perceive the kind of fruit mashed, because the strong stone aroma does not cover the delicate, fruit typical components. Sieving and destoning machines are available allowing a simple removal of the stones (Jung 2003). In this work, the use of the so-called 'complete cherry mash' was demonstrated towards the stoneless mashes. On a small scale this low-cost machine allows the separation of the fruit flesh from the stones and simultaneously makes a homogeneous mash. Dependent on the time of the separation, distillates with a subtle bitter almond aroma but with distinct fruit flavour emerge (Hagmann 2002). Worth mentioning is the fact that the stones stay undamaged during the process (Senn and Jung 1999). The results of our experimental production of stone-fruit spirits demonstrate in striking difference to the commercial samples that the production of ethyl carbamate-free spirits is possible even for small distilleries.

Conclusion

The results show that nearly 20 years after the first warnings about ethyl carbamate in spirit drinks, the problem especially persists in products from small distilleries. Even if the intake cannot be completely avoided because of its natural occurrence in all kinds of fermented foods and beverages, we showed that using state-of-the-art technologies, the occurrence of ethyl carbamate in stone fruit spirits can be prevented. Even for small distilleries, simple possibilities like destoning or process control using cyanide test-kits exist to minimize the ethyl carbamate content.

Acknowledgments

The authors commend S. Gonzalez for her faithful and long-standing work in ethyl carbamate analysis since 1986, without which this work had not been possible. H. Heger, M. Trautner and H. Havel are thanked for excellent technical assistance.

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