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ETHYL CARBAMATE FORMATION IN THE PRODUCTION OF POT STILL WHISKY

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The formation and distribution of ethyl carbamate (urethane) during pot still distillation was investigated. Formation only occurred if the distillation was carried out in the presence of copper. Removal or chelation of dissolved or suspended copper prevented ethyl carbamate formation. When copper was present, during and subsequent to distillation, formation of ethyl carbamate was time-dependent. The degree of formation was maximised between pH 4 and 6. During the second or low wines distillation only 1-2 per cent of the total available ethyl carbamate was collected with the potable spirit fraction. The remainder was distributed between the feints (15 per cent) and the spent lees (84 per cent).

Key Words: *Ethyl carbamate, distillation, pot still whisky, copper, potable spirit.*

INTRODUCTION

Ethyl carbamate, also known as urethane, has been detected at trace levels (<10 ppb) in many foods and beverages where microbiological activity has been an integral part of the production process[1,5,6,7]. This activity confers natural flavouring and preservative characteristics on products such as beers, wines, cheeses and yoghurts. When fermented beverages are distilled, the natural levels of ethyl carbamate are found to increase[4].

Elevation of natural levels of ethyl carbamate, subsequent to distillation, has been shown to be particularly prevalent in stone fruit brandies, where amounts averaging 1 500 ppb have been reported[9].

The significance of ethyl carbamate in fermented foods and beverages and in potable spirits relates to the toxicological aspects of the compound. Ethyl carbamate has been implicated as a carcinogen in laboratory rats and mice, where daily dose levels in excess of 100 μg per kg body weight resulted in an apparent increase in the incidence of death by malignant tumours or leukemia[8]. An increase in daily dose up to 12 500 μg per kg body weight resulted in a rise in the number of dead animals with tumours. Mice appeared more susceptible than rats with the latter able to tolerate up to 500 μg per kg per day before significant increases in malignant tumours became apparent. However, this study[8] failed to distinguish between tumours of biological significance and the total number of tumours present. Accordingly, this may mean that the results are suspect in respect of risk assessment purposes for laboratory rodents and substantially invalid if they are extrapolated as a potential risk evaluation for ethyl carbamate carcinogenesis in man.

In November 1985 the Health Protection Branch of the Department of Health and Welfare Canada published the following maximum acceptable levels for ethyl carbamate in fermented beverages and distilled spirits (ppb): wine, 30; fortified wine, 100; distilled spirits, 150 and liqueurs and fruit brandies, 400. These levels were based on reported differing consumption patterns and immediately became the guidelines used by all the Provincial Liquor Boards. The methods for determining the maximum acceptable levels have been described by Conacher and Page[2] and were based on a calculated tolerable daily intake for humans of 0.3 μg per kg body weight. Presently, action levels for distilled spirits of 125 ppb are being considered in the United States while the Swiss have proposed 400 ppb.

There is general agreement that ethyl carbamate levels in fermented beverages and distilled spirits should be maintained at the lowest levels that are technically possible.

Clearly, in order to attain this goal it is essential that the mode of formation be properly understood. This report describes a number of experiments and results which define how ethyl carbamate is formed and distributed during the traditional double distillation procedure[3] as used in the manufacture of pot still whisky.

METHODS

Distillation

Fermented wort (wash), prepared from 100 per cent barley malt mashes and fermented by the addition of yeast only, was distilled in either laboratory copper stills (alembic-type stills) or in all-glass distillation apparatus. Three distillation systems were used.

1. Two litre capacity all-copper wash still with the following dimensions: pot base (cylindrical), diameter 15 cm, height 11 cm; pot shoulders (conical), base diameter 15 cm rising through 11 cm to an apex diameter of 6 cm; head (conical), base diameter 6 cm rising through 13 cm to an apex diameter of 2 cm; lyne arm (downwards angle of 30°), diameter 2 cm, length 28 cm and connected to a Thorpe 1761/02, 8-spiral condenser with an overall length of 35.5 cm. The still was heated directly with a bunsen flame and the average distillation rate was 4.4 ml per min.
2. 700 ml capacity all-copper low wines still with the following dimensions: pot base (cylindrical), diameter 10 cm, height 10 cm; pot shoulders (conical), base diameter 10 cm rising through 6 cm to an apex diameter of 5 cm; head (conical), base diameter 5 cm rising through 7.5 cm to an apex diameter of 2 cm, lyne arm (downwards angle of 40°), diameter 2.0 cm, length 33 cm and connected to a Thorpe 1761/02, 8 spiral condenser with an overall length of 35.5 cm. The still was heated directly with a bunsen flame and the average distillation rate was 2.0 ml per min.
3. All-glass distillation apparatus with the following components (quickfit): round bottom flask (3 litre 24/29), sloping splash head (24/29) and Thorpe 1761/02, 8 spiral condenser with an overall length of 35.5 cm. The still was heated directly with a bunsen flame and the average distillation rate was 4.4 ml per min.

Analysis of ethyl carbamate

Sample preparation

Following determination of alcoholic strength by volume, a sample of the distillate was diluted to 10 per cent v/v alcohol by the addition of an appropriate amount of 10 mM di-sodium tetraborate buffer (pH 9.2). An aliquot (10 ml) of the diluted solution was measured into a separating funnel (100 ml) with the addition of sodium chloride (1 g). Propyl carbamate (100 μl) at a concentration of 1.6 μg per ml was added to the separating funnel as the internal standard. The funnel was then gently swirled until the salt dissolved and the contents were then extracted by manual shaking for 90 s with 3 × 15 ml of dichloromethane (DCM). The DCM washings were bulked and dried by passing through a small column (0.5 × 10 cm) containing anhydrous sodium sulphate (0.25 g). The dried DCM extract was reduced in volume to about 1 ml on a rotary

film evaporator and transferred to a small vial where a final evaportion to about 200 μl was carried out under a gentle stream of nitrogen gas.

Determination of ethyl carbamate

An aliquot (1 μl) of the final extract was applied to a Hewlett-Packard 5890A Gas Chromatograph which was linked to a Trio-2 VG Masslab Automated Mass Spectrometer. The injection was vapourising-splitless with an injector temperature of 200°C. The chromatographic column was a 0.20 μm Hewlett Packard FFAP capillary column (25 M), programmed to run from 60-195°C at a temperature gradient of 5°C per min. The carrier gas was helium at a flow rate through the column of 20 cm per s. The total run time was 25 min. Peak detection was by single-ion monitoring mass detection at 62 m/z.

A calibration curve was obtained by direct injection of a series of ethyl carbamate/DCM standards over a concentration range of 0-2.0 mg per litre ethyl carbamate. Propyl carbamate (internal standard) at a concentration of 0.8 mg per litre was included with each injection. Each standard was injected 4 times. The resulting data was computed by linear regression and used subsequently to determine the ethyl carbamate concentrations in the DCM extracted samples.

Reproducibility of the method of analysis was determined by repeat injections (4) of sample extracts containing ethyl carbamate in varying amounts (45, 110 and 175 ppb). The coefficient of variation (cv) was calculated for each sample set.

Extraction recovery rates were determined by analysing a sample of neutral grain spirit (40 per cent v/v alcohol) containing added ethyl carbamate to a concentration of 100 ppb. An aliquot (2.5 ml) of the spiked sample was prepared, in duplicate, for ethyl carbamate analysis as described previously. Recovery percentages were determined, with reference to the analysis of a control sample derived similarly but without added ethyl carbamate.

Determination of ethanol concentration

The concentration of ethanol in the samples of distillate was measured by density measurements using a Stanton Redcroft Paar DMA 55 calculating density meter.

Determination of dissolved copper content

Levels of dissolved copper in the fractions of distillate were determined directly by atomic absorption in an air-acetylene flame at 324.8 nm using a Varian Model 1100 Atomic Absorption Spectrophotometer at a slit width of 0.5 nm.

A calibration curve was prepared by direct aspiration of a series of copper nitrate standards over a range of 0-30 mg per litre in 20 per cent v/v ethanol. When the ethanol concentration in the samples differed significantly from 20 per cent v/v, additional calibration curves were prepared at appropriate ethanol concentrations. All standards and samples were analysed in duplicate.

Ion exchange

Amberlite IRC 718 ion exchange resin (50 g) was washed in distilled water (6 × 250 ml) and an aliquot (~20 ml) of the final rinsed slurry poured into a small glass column (15 × 360 mm) containing a sintered glass disc for resin retention. The settled resin dimensions were 12 × 140 mm giving a bed volume of 15.83 cm^3. The column was converted to H^+ by eluting with 100 ml of 2 per cent v/v hydrochloric acid at a rate of 5 ml per min. Excess hydrochloric acid was removed by eluting with distilled water (200 ml) at 5 ml per min. The efficiency of hydrochloric acid elution was monitored by measuring the pH of the eluate. The column was then equilibrated by eluting with fresh copper distilled low wines (100 ml) at 5 ml per min. A further 100 ml of the low wines were then eluted through the column and the eluate collected and estimated for ethyl carbamate concentration and copper content.

TABLE I. Analysis of ethyl carbamate — method reproducibility and recovery data

1a. Reproducibility data

Sample set (ppb EC)	Ethyl carbamate analysis (ppb)	Coefficient of variation (%)
45	48	
45	47	6·4
45	46	
45	53	
110	95	
110	96	3·8
110	89	
110	90	
175	180	
175	179	2·1
175	178	
175	173	

1b. Recovery data

Sample (ppb EC)	Control analysis (ppb EC)	Sample analysis (ppb EC)	Recovery %
100	ND	92	92
100	ND	88	88
100	ND	94	94
100	ND	91	91

Key: EC = ethyl carbamate
ND = non-detectable

Results

The method reproducibility and recovery data are given in tables 1a and 1b. The reproducibility of the sample injections was found to improve as the ethyl carbamate concentrations tended towards the upper range of the calibration curve. Additionally, the data, at the 3 concentration levels, compute individually to 95 per cent confidence limits of ± 6 ppb. The recovery data imply that the general levels of ethyl carbamate as determined by the extraction procedures, may have been underestimated by approximately 10 per cent.

TABLE II. Formation of ethyl carbamate in low wines as a function of time after distillation

Time after distillation (h)	Ethyl carbamate in low wines (ppb)	
	Copper distilled	Glass distilled
0	10	<5
2	12	<5
4	18	<5
6	23	<5
8	34	<5
24	49	<5
30	55	<5
48	60	<5

Table 2 shows the determined levels of ethyl carbamate, as a function of time after distillation, in 2 samples of low wines. Both samples were obtained from distillations of fermented wash with a pre-distillation alcoholic strength of 7.5 per cent v/v. The strengths of the low wines (800 ml per distillate) samples were 19.0 per cent v/v alcohol. When the copper distilled low wines were sampled and determined for ethyl carbamate levels, the data show that ethyl carbamate formation was time dependent. The samples analysed from the glass distillate gave ethyl carbamate levels of <5 ppb

regardless of the time elapsed before analysis. Both these samples of low wines were kept at ambient temperatures throughout the period of sampling.

In a second experiment, low wines (800 ml) obtained from a copper wash still distillation were analysed for time dependent ethyl carbamate formation at (1) ambient temperatures and (2) subsequent to heating in glass at 50°C for 20 min. The results are given in table 3. The data clearly shows that heating of the freshly distilled low wines eliminates, essentially, the observed time dependent feature of ethyl carbamate formation.

TABLE III. Effect of time and temperature on ethyl carbamate formation in low wines

Time after distillation (h)	Ethyl carbamate in low wines (ppb)	
	Ambient	Heated (50° for 20 min)
0	18	105
4	30	103
8	75	106

Both sets of data, given in tables 2 and 3, were derived from single extractions but duplicate injections. The results shown are the mean values of these duplicate injections which were within the control parameters shown previously in table 1. The remaining data given in this work were obtained from similar determinations.

In order to investigate further a possible copper-dependent effect on ethyl carbamate formation, a third sample of low wines (800 ml) was prepared as described in methods. On completion of distillation, an aliquot (5 ml) of low wines (alcohol strength 19.0 per cent v/v) was immediately analysed for ethyl carbamate content while a large aliquot (100 ml) was treated with EDTA to a concentration of 10 mM. The remaining distillate was divided into 2 portions with the first kept at room temperature in the dark, while the second was subjected to artificial light. The 3 samples were all analysed for ethyl carbamate content following a 72 h time interval. The results are shown in table 4.

TABLE IV. Effect of EDTA on ethyl carbamate formation

Time after distillation (h)	Ethyl carbamate in low wines (ppb)
0	9
72	162
72 (EDTA)	12
72 (Dark)	147

The addition of EDTA was found to inhibit ethyl carbamate formation, with the levels essentially maintained at those of the control sample. In the absence of EDTA, the levels reached 162 ppb after 72 h. There was a slight reduction in ethyl carbamate formation for the sample kept in the dark.

The previous experiment was repeated except that the EDTA treated sample was redistilled in copper (as described in methods) and then re-analysed for ethyl carbamate formation. The time elapsed period was 24 h. The results from this experiment are given in table 5. In the absence of EDTA, the sample analysed at 166 ppb ethyl carbamate after 24 h. When the EDTA treated sample was redistilled in copper the distillate showed time-dependent formation similar to that observed for the non EDTA treated control.

The observed copper-dependent effect was studied further by passing a sample of fresh copper distilled low wines through an ion-exchange column as described in methods. The concentration of copper in the distillate before and after ion exchanging was measured as were the levels of ethyl carbamate. The results are given in table 6 and indicate that the ion exchange procedure inhibited completely the previously observed time dependent formation of ethyl carbamate.

TABLE V. Effect of redistillation (in copper) of EDTA treated low wines

5a. First distillation (production of low wines)

Time after distillation (h)	Ethyl carbamate in low wines (ppb)
0	12
24	166
24* (EDTA)	12

5b. *24 (EDTA) redistilled in copper

Time after distillation (h)	Ethyl carbamate in redistilled low wines (ppb)
0	12
2	30
24	144

TABLE VI. Ethyl carbamate levels in cation — exchanged low wines

Sample	Time after distillation (h)	Ethyl carbamate (ppb)	Copper (ppm)
Control LW	0	15	0·88
Control LW	24	47	0·88
Cation exchanged LW	0	14	ND
Cation exchanged LW	24	15	ND

Key: LW = low wines
ND = non-detectable

In order to determine, whether the cation exchange resin had taken out substances, other than copper, responsible for ethyl carbamate formation, a sample of cation exchanged low wines was redistilled in copper. This sample was obtained by passing a further aliquot (325 ml) of low wines through the ion exchange column. This aliquot was then redistilled in the low wines still as described in methods. The distillate (170 ml) and the residue (155 ml) were analysed for ethyl carbamate and copper and the results are given in table 7.

TABLE VII. Distallation of cation exchanged low wines in copper

Sample/Volume (ml)	Ethyl carbamate (ppb)	Copper (ppm)
*Cation exchanged LW/325	15	ND
Distillate/170	14	4·3
Residue/150	60	>30·0

Key: * Cation exchanged low wines prior to distillation

The data show that, on redistillation in copper, ethyl carbamate was formed although the bulk of the ethyl carbamate (~80 per cent) was retained in the residue.

The pH-dependency of ethyl carbamate formation in low wines was investigated by preparing a sample of low wines (800 ml) as described in methods. On completion of distillation, the low wines were thoroughly mixed and split into 10 equal aliquots (80 ml) and pH-adjusted using hydrochloric acid or sodium hydroxide. Following pH-

adjustment, each aliquot was made up to 100 ml with distilled water and, following 24 h standing at ambient temperatures, determined for ethyl carbamate levels as described in methods. The results are shown in fig. 1 and show a clear maximisation of ethyl carbamate formation between pH 4 and 6. The pH of the low wines immediately following distillation was 4.2.

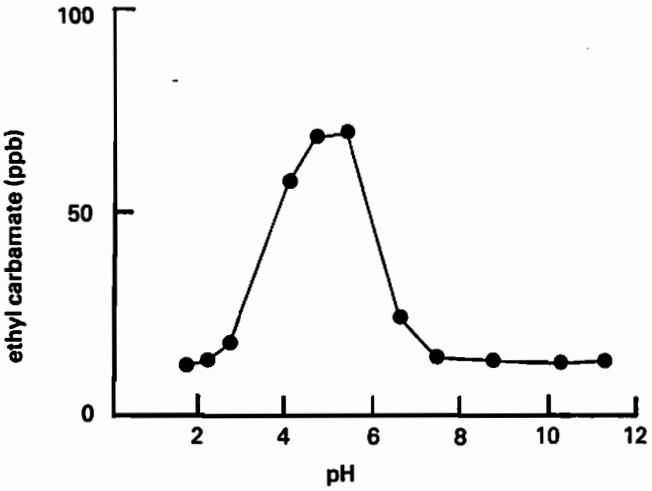

FIG. 1. Ethyl carbamate formation in low wines distillate as a function of pH.

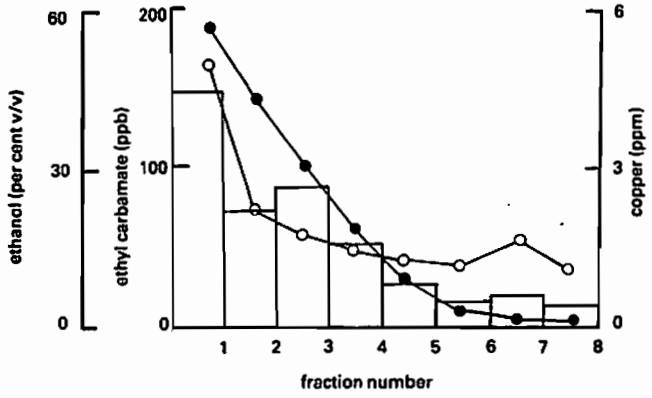

FIG. 2. Laboratory copper still wash distillation.

Distillate (low wines) collected in fractions (100 ml) and each fraction was analysed for ethyl carbamate (histogram), ethanol (●) and copper (○).

Figs. 2 and 3 show the distribution of ethyl carbamate during both the wash distillation and the second distillation (low wines distillation). In both cases the distillate was collected as discrete fractions as it condensed into a receiving vessel. Ethyl carbamate analysis was carried out as described in methods, following 24 h standing at ambient temperatures.

For the wash distillation, each fraction was 100 ml, while the fraction sizes were varied during the low wines distillation (see fig. 3.) This latter procedure was carried out to demonstrate the critical points of the low wines distillation, relative to the traditional process used in the manufacture of pot still whisky. The graphs also show the values for ethanol concentration and copper content in each fraction.

During the distillation of fully fermented wash (fig. 2), formation of ethyl carbamate (subsequent to 24 h time elapse) was highest in the early fractions i.e. where alcohol and copper concentrations were also at their highest amounts. As the alcohol concentration dropped, the later fractions were observed to have reduced ethyl carbamate content.

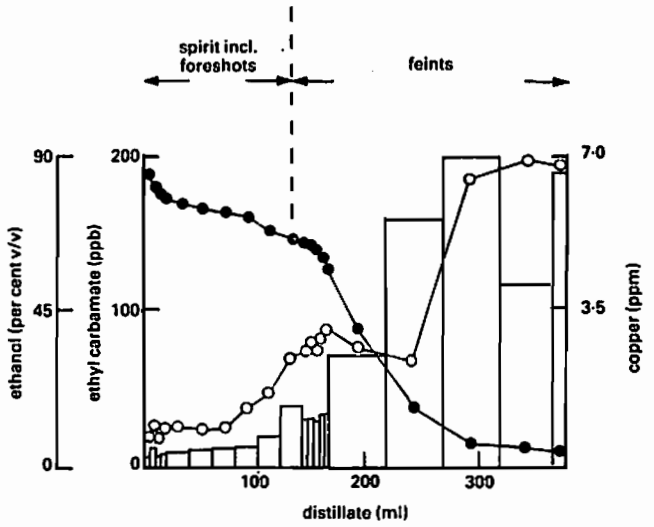

FIG. 3. Laboratory copper still low wines distillation.

Distillate collected in various fraction sizes as follows (fraction no., ml):
1-4, 5; 5-10, 20; 11-15, 5; 16-19, 50 and 20, 10.
Each fraction was analysed for ethyl carbamate (histogram), ethanol (●) and copper (○).

When low wines were redistilled in the laboratory low wines still (fig. 3), the amounts of ethyl carbamate in the collected fractions increased as the alcohol strength dropped. If the amounts of ethyl carbamate present in the various fractions were expressed as a precentage of the total, including the residue (spent lees), it was found that 1 per cent of the total was distilled during the traditional spirit cut while 15 per cent was found in the feints. Approximately 84 per cent was retained in the spent lees.

DISCUSSION

Elevation of natural ethyl carbamate levels, subsequent to distillation, only occured if the distillation was carried out in the presence of copper. When fermented worts were distilled in all-glass distillation apparatus there was no significant ethyl carbamate formation.

This work demonstrates that not only is copper essential for ethyl carbamate formation, but that the mechanism of formation is dependent upon hot copper contact with some precursor which is apparently present in the vapour phase of the process.

The time-dependent aspect of ethyl carbamate formation implicates the formation of the precursor compound during distillation since ethyl carbamate is not formed directly but occurs subsequent to distillation. Since either the presence of EDTA in the distillate or cation-exchanging the fresh distillate, both prevented ethyl carbamate formation, this implies that the active precursor compound either incorporates copper or requires copper to catalyse its ethanolysis.

The second or low wines distillation apparently eliminates the time-dependent factor and ethyl carbamate formation proceeds as soon as sufficient heat is applied to the still. Fortuitously ethyl carbamate, once formed, does not readily distil when the alcohol concentration is high. This means that only a small proportion, approximately 1-2 per cent of the total available ethyl carbamate, will distil over during collection of potable spirit. As the alcohol strength drops from 64 per cent v/v to about 1.5 per cent v/v, a further 15 per cent of the available ethyl carbamate will be collected with the feints. Clearly, the elimination of 84 per cent of the total available ethyl carbamate with the spent lees prevents substantial accumulation in the feints. This is a significant feature of the current work, since the practice within the pot still whisky industry is to recycle the feints.

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