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The role of acetaldehyde outside ethanol metabolism in the carcinogenicity of alcoholic beverages: Evidence from a large chemical survey

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ABSTRACT

Acetaldehyde is a volatile compound naturally found in alcoholic beverages, and it is regarded as possibly being carcinogenic to humans (IARC Group 2B). Acetaldehyde formed during ethanol metabolism is generally considered as a source of carcinogenicity in alcoholic beverages. However, no systematic data is available about its occurrence in alcoholic beverages and the carcinogenic potential of human exposure to this directly ingested form of acetaldehyde outside ethanol metabolism. In this study, we have analysed and evaluated a large sample collective of different alcoholic beverages ($n = 1555$). Beer (9 ± 7 mg/l, range 0–63 mg/l) had significantly lower acetaldehyde contents than wine (34 ± 34 mg/l, range 0–211 mg/l), or spirits (66 ± 101 mg/l, range 0–1159 mg/l). The highest acetaldehyde concentrations were generally found in fortified wines (118 ± 120 mg/l, range 12–800 mg/l). Assuming an equal distribution between the beverage and saliva, the residual acetaldehyde concentrations in the saliva after swallowing could be on average 195 μM for beer, 734 μM for wine, 1387 μM for spirits, or 2417 μM for fortified wine, which are above levels previously regarded as potentially carcinogenic. Further research is needed to confirm the carcinogenic potential of directly ingested acetaldehyde. Until then, some possible preliminary interventions include the reduction of acetaldehyde in the beverages by improvement in production technology or the use of acetaldehyde binding additives. A re-evaluation of the 'generally recognized as safe' status of acetaldehyde is also required, which does not appear to be in agreement with its toxicity and carcinogenicity.

1. Introduction

Acetaldehyde (ethanal, CH_3CHO) is a potent volatile flavouring compound found in many beverages and foods (Liu and Pilone, 2000). Acetaldehyde at low levels gives a pleasant fruity aroma, but at high concentrations it possesses a pungent irritating odour (Miyake and Shibamoto, 1993). In alcoholic beverages, acetaldehyde may be formed by yeast, acetic acid bacteria, and coupled auto-oxidation of ethanol and phenolic compounds (Liu and Pilone, 2000). Acetaldehyde is extremely reactive and readily binds to proteins, specifically to the peptide glutathione or to individual amino acids to generate various flavour compounds (Liu and Pilone, 2000; Miyake and Shibamoto, 1993).

According to the International Agency for Research on Cancer (IARC), there is sufficient evidence in animals to demonstrate carcinogenicity of acetaldehyde and therefore it is possibly carcinogenic to humans also (Group 2B) (IARC, 1999). In a recent IARC meeting, acetaldehyde was discussed in the context of the carcinogenicity of alcoholic beverages. The IARC working group agreed that substantial mechanistic evidence in humans deficient in aldehyde dehydrogenase (ALDH) indicates that acetaldehyde derived from the metabolism of ethanol in alcoholic beverages contributes to causing malignant oesophageal tumours (Baan et al., 2007; IARC, in press). Acetaldehyde is able to cause point mutations or to form covalent bonds with DNA, leading to carcinogenesis (Cheng et al., 2003; Fang and Vaca, 1997; Hecht et al., 2001; Noori and Hou, 2001; Wang et al., 2000). Recent experimental evidence shows that acetaldehyde can form mutagenic adducts in cellular concentrations of 100 μM and above (Theruvathu et al., 2005). This is in accordance with findings in man, which show that salivary acetaldehyde concentrations after a moderate dose of alcohol range between 18 and 143 μM within 40 min of alcohol ingestion (Homann et al., 1997a). The mutagenic and carcinogenic changes caused by acetaldehyde can already occur with an acetaldehyde concentration from 40 to 200 μmol/l (Homann et al., 1997a; Salaspuro et al., 2002).

Furthermore, acetaldehyde interferes with DNA repair mechanisms by inhibiting repair enzymes (Espina et al., 1988). Additionally, genetic epidemiological studies provide strong evidence that the heterozygous genotype (ALDH2*1/*2) contributes substantially to the development of oesophageal cancer related to alcohol consumption, with up to a 12 fold increase in risk for heavy drinkers in comparison to carriers of the homozygous ALDH2*1/*1 genotype (which encodes the active enzyme) (Lewis and Smith, 2005).

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ALDH deficient humans have higher levels of acetaldehyde in their blood (Mizoi et al., 1979) and saliva (Väkeväinen et al., 2000) after drinking alcohol, and according to a recent study higher levels of acetaldehyde-related DNA adducts have been measured in their lymphocytes (Matsuda et al., 2006). After ingestion of a moderate dose of alcohol, salivary acetaldehyde concentrations are 2–3 times higher among ALDH2-deficient subjects than in those with the normal ALDH2 enzyme, which is associated with a remarkably increased risk for digestive tract cancers (Salaspuro, 2003b; Väkeväinen et al., 2000).

In addition to acetaldehyde metabolism in the gastrointestinal tract and in the liver, the oral and colonic bacterial flora may considerably contribute to an accumulation of acetaldehyde (Homann et al., 1997a,b; Homann, 2001; Jokelainen et al., 1996a,b; Kurkivuori et al., 2007; Salaspuro, 2003a; Väkeväinen et al., 2000, 2001). For this reason, poor dental status or lacking oral hygiene are associated with a higher risk for cancer of the upper gastrointestinal tract (Homann et al., 2000a,b, 2001). In addition, chronic alcohol abuse may lead to atrophy of the parotid glands and reduced saliva flow, which aids local acetaldehyde accumulation (Salaspuro, 2003b).

In summary, the IARC working group confirmed that alcoholic beverages are 'carcinogenic to humans' (Group 1) and concluded that the occurrence of malignant tumours of the oral cavity, pharynx, larynx, oesophagus, liver, colorectum, and female breast is causally related to alcohol consumption (Baan et al., 2007; IARC, in press).

During the IARC meeting, an absence of information about acetaldehyde outside ethanol metabolism was noted. There are indications that consumption of spirits with exceptionally high concentrations of acetaldehyde might lead to an increased risk for cancer of the oesophagus (Linderborg et al., 2008). However, there are no systematic and actual data available about the occurrence of acetaldehyde in alcoholic beverages to evaluate its carcinogenic potential.

In this study, we collected novel data on the acetaldehyde content of a large collection of different alcoholic beverages (over 1500 samples). The data was statistically evaluated to find differences between sub-groups (i.e. beer, wine, fortified wine and spirits), as well as to estimate the typical ingested amount of acetaldehyde and its possible concentrations in saliva after ingestion. Finally, we provide a risk analysis for acetaldehyde outside ethanol metabolism and propose intervention measures.

2. Material and methods

2.1. Samples

Between January 2000 and March 2008, 1555 alcoholic beverages submitted to the CVUA Karlsruhe were routinely analysed for acetaldehyde. Our institute covers as a part in official food control the district of Karlsruhe in North Baden (Germany), which has a population of approximately 2.7 million. The samples were randomly selected either directly at the breweries, distilleries and wineries or from retail trade by governmental food inspectors. The samples were stored at 8 °C in the original bottles, which were not opened prior to the analysis.

2.2. Analytical procedure

All samples were analysed for alcoholic strength and acetaldehyde on the basis of the European Community reference methods for the analysis of spirits (European Commission, 2000). The alcoholic strength was obtained from the density of the distillate measured with the oscillation-type density meter DE51 by Mettler-Toledo (Giessen, Germany) as outlined in Lachenmeier et al. (2003, 2005a). The acetaldehyde content in extract-free alcoholic beverages like vodka, whisky, brandy, rum, wine spirit, fruit spirit, calvados or grape marc spirit was determined by direct injection into the gas chromatographic (GC) system. Acetaldehyde in beverages with considerable amounts of total dry extract was distilled prior to injection into the GC system (Frank, 2002). The GC system used for analysis was a Trace 2000 gas chromatograph (Thermo Electron Scientific Instrument Division, Dreieich, Germany). Data acquisition and analysis were performed using the Chromeleon Chromatography Information Management System (Dionex, Idstein, Germany). Substances were separated on the fused silica capillary column CP-WAX 52CB, 60 m × 0.32 mm I.D., film thickness 0.5 μm (Varian Deutschland GmbH, Darmstadt, Germany). Temperature program: 40 °C hold for 15 min, 4 °C/min up to 200 °C, hold for 10 min, 15 °C/min up to 230 °C, hold for 10 min. The temperature for the injection port was set at 260 °C. After addition of an internal standard (*n*-amyl alcohol), the samples were injected using split injection mode (2 μl, 1:5) and helium with a constant flow rate of 6.5 ml/min as carrier gas.

2.3. Indication of results

The volatile compounds of alcoholic beverages are primarily calculated and expressed in the unit 'g/hl of pure alcohol' or 'g/hl of 100% vol alcohol' (i.e. the concentrations are standardized in regard to the alcoholic strength) according to the demands in the European Community reference methods for the analysis of spirits (European Commission, 2000). This has the advantage that high-proof distillates and distillates diluted to drinking strength can be directly compared. For better readability, the following text uses the abbreviation 'g/hl p.a.'.

We also give the results recalculated to 'mg/l' and 'μmol/l', as these units are preferred in the medical literature. Finally, we calculated the acetaldehyde amount in μg found in a standard portion of each type alcoholic beverage (beer and apple wine (250 ml), wine (120 ml), fortified wine (90 ml), spirit (30 ml)). The volume of each standard drink was estimated on the basis of an evaluation of Turner (1990).

2.4. Calculation of acetaldehyde increase in saliva

To evaluate the risk of directly ingested acetaldehyde, the following model calculation was conducted. We assume that after drinking of a swallow of any alcoholic beverage, the acetaldehyde will be equally distributed between the beverage and the volume of saliva in the mouth before swallowing, which is 1.1 ml according to Lagerlöf and Dawes (1984). The mean bolus volume of 26 ml according to Nilsson et al. (1996) was used for wine and beer, whereas for fortified wines and spirits a mean bolus volume of 10 ml was assumed. Therefore, the acetaldehyde concentration in the beverage/saliva mixture is diluted by a factor of 0.95 (wine, beer) or 0.90 (fortified wines, spirits). After swallowing, a residual saliva volume of 0.8 ml remains in the oral cavity (Lagerlöf and Dawes, 1984).

2.5. Statistics

All data were evaluated using Origin V7.5 (Originlab, Northampton, USA). Statistical significance was assumed at below the 0.05 probability level. 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. Box and whisker plots were used for visualization of data (box 25th–75th percentile, line in the box: median, whiskers: minimum and maximum (max. 1.5 times the length of the inner quartiles), data points outside are outliers).

3. Results

The determined acetaldehyde levels in alcoholic beverages are presented in Table 1.

For beer and wine no significant differences were found in sub-groups (e.g. bottom- and top-fermented beers, red and white wine). In the fortified wines, sherry had significantly higher acetaldehyde concentration than port wine or other fortified wines (including Madeira, Marsala and some fortified wines from Greece and Eastern Europe). The spirit groups also showed significant differences. For example, the lowest acetaldehyde content was found in vodka, higher concentrations were found in rum, whisky, brandy and fruit spirits, and the highest in Bacanora from Mexico and some Chinese spirits.

The acetaldehyde contents of the main groups of alcoholic beverages are compared in Figs. 1–3. If the acetaldehyde contents are standardized to the alcoholic strength (Fig. 1), fortified wines have higher acetaldehyde contents than all other groups, whereas between beer, wine and spirits no significant differences exist. In general, we found no significant correlation between acetaldehyde and alcoholic strength.

If we look at the concentrations calculated in mg/l (Fig. 2), beer has a significantly lower concentration than all other groups and fortified wines again show the highest concentrations. The picture changes if acetaldehyde per standard drink is calculated (Fig. 3). An average standard drink of beer and wine contains more

Table 1
Acetaldehyde in alcoholic beverages (SD = standard deviation)

Group of beverage (origin if known)	*n*	Alcoholic strength (% vol)	Acetaldehyde (g/hl p.a.)		Acetaldehyde (mg/l)		Acetaldehyde (μmol/l)		Acetaldehyde (μg/standard drink)[b]	
		Mean, SD	Mean, SD	Min Max	Mean, SD	Min Max	Mean, SD	Min Max	Mean, SD	Min Max
Beer (Germany)	364	5.2 ± 0.9	18 ± 14	0–156	9 ± 7	0–63	205 ± 150	0–1435	2257±1653	0–15824
Wine (Europe)	213	12.3 ± 1.4	28 ± 28	0–207	34 ± 34	0–211	773 ± 760	0–4780	4092±4023	0–25298
Fortified wines – All (Europe)	133	16.4 ± 2.1	76±82	7–601	118 ± 120	12–800	2686±2728	268–18139	10671 ± 10821	1065–71994
– Port (Portugal)	27	19.2±1.3	50 ± 111	12–601	84 ± 146	22–800	1909 ± 3306	505–18139	7577±13122	2003–71994
– Sherry (Spain)	53	15.0 ± 0.8	104 ± 73	29–347	156±109	50–523	3537 ± 2482	1132–11876	14038 ± 9850	4495–47136
– Other fortified wines (Europe)	53	16.2±1.9	60 ± 63	7–377	98 ± 108	12-647	2231±2450	268–14670	8879 ± 9713	1065–58223
Apple wine/Cider (Germany, France)	11	5.3 ± 1.0	97 ± 80	24–253	50±41	15–133	1123 ± 932	343–3007	12376 ± 10278	3778–33149
Spirits – All (Worldwide)	834	41.1 ± 7.7	17±25	0–293	66 ± 101	0–1159	1541 ± 2344	0–26280	2038±3101	0–34769
– Vodka (Europe)	72	39.0 ± 1.1	0.7 ± 0.7	0–3	3 ± 3	0–13	61 ± 70	0–287	81 ± 92	0–380
– Rum	38	41.6 ± 6.5	4 ± 3	0–17	18 ± 14	0–68	403 ± 321	0–1548	533 ± 425	0–2047
– Whisky (Scotch, Irish, Bourbon)	37	40.1 ± 1.1	7 ± 5	0–19	28 ± 20	0–77	627 ± 448	0–1763	829 ± 593	0–2332
– Brandy/cognac (Germany, France)	82	36.6 ± 2.1	20 ± 13	0–59	75 ± 48	0–211	1704 ± 1096	0–4776	2254 ± 1451	0–6319
– Fruit spirits/marc spirits (Germany)	315	40.8 ± 2.1	21 ± 29	0–293	86 ± 119	0–1159	1953 ± 2704	0–26280	2638 ± 3631	0–34769
– Calvados (France)	27	40.1 ± 1.4	9 ± 4	4–16	38 ± 15	19–67	870 ± 334	437–1524	1152 ± 442	578–2016
– Cachaça (Brazil)	21	39.7 ± 0.5	13 ± 5	6–30	51 ± 22	24–120	1149 ± 491	537–2716	1521 ± 650	711–3593
– Tequila (Mexico)[a]	70	42.8 ± 8.3	15 ± 23	0–191	60±86	0–670	1371 ± 1960	0–15188	1814 ± 2594	0–20094
– Mezcal (Mexico)[a]	10	42.4 ± 6.2	20 ± 18	1–50	93 ± 89	4–241	2103 ± 2024	88–5476	2784 ± 2678	117–7244
– Sotol (Mexico)[a]	16	39.9 ± 3.8	21 ± 15	0–52	83 ± 59	0–196	1876 ± 1346	0–4454	2482 ± 1781	0–5892
– Bacanora (Mexico)[a]	13	46.8 ± 2.5	73 ± 48	7–147	340 ± 223	33–696	7711 ± 5061	752–15779	10201 ± 6696	995–20876
– Chinese spirits	30	49.9 ± 14.3	62 ± 24	23–116	327 ± 174	33–721	7419 ± 3955	755–16343	9815 ± 5232	999–21622

[a] Data taken from a previous investigation (Lachenmeier et al., 2006).
[b] The following portions were used as 'standard drink': beer and apple wine (250 ml), wine (120 ml), fortified wine (90 ml), and spirits (30 ml).

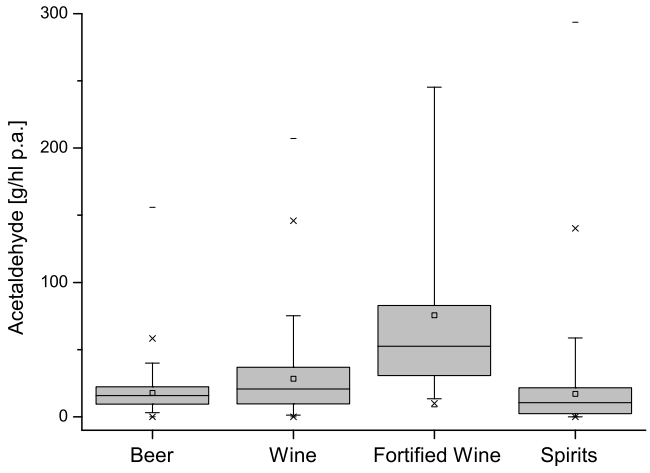

Fig. 1. Box chart of the acetaldehyde content of alcoholic beverages (in g/hl p.a.).

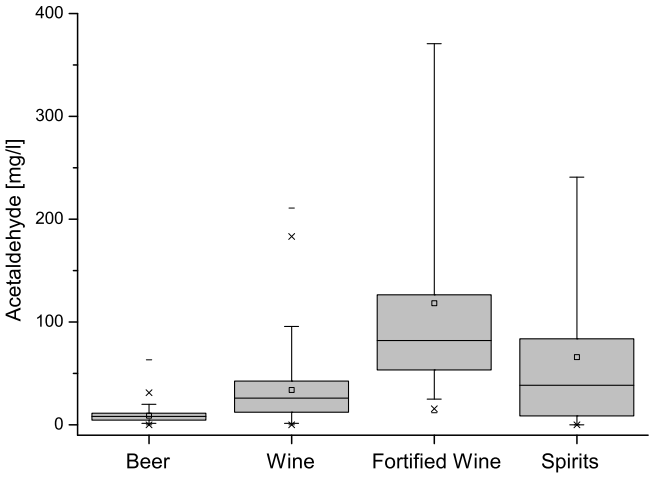

Fig. 2. Box chart of the acetaldehyde content of alcoholic beverages (in mg/l).

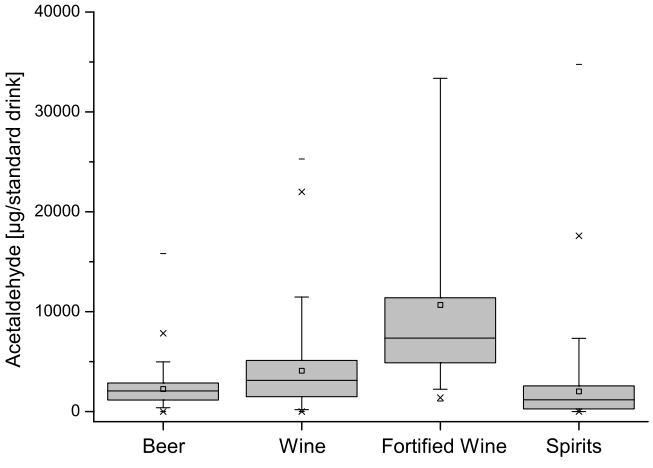

Fig. 3. Box chart of the acetaldehyde content of alcoholic beverages (in µg/portion).

acetaldehyde than one drink of spirits, whereas fortified wine again contains the highest acetaldehyde concentration in this regard.

The resulting increase in saliva concentration of acetaldehyde after ingestion is shown in Table 2. It should be noted that the amount of acetaldehyde from metabolism is not included in this calculation, so that the values must be interpreted as the possible increase in acetaldehyde concentration by the directly ingested content in the beverages.

Table 2
Possible acetaldehyde concentrations in residual saliva after swallowing one mouthful of alcoholic beverage

µmol/l	Mean	SD	Min	Max
Beer	195	142	0	1363
Wine	734	722	0	4541
Fortified wine	2417	2455	241	16325
Spirits	1387	2110	0	23652

Theoretical calculation based on acetaldehyde concentrations found in the beverages; metabolic acetaldehyde was not regarded.

4. Discussion

4.1. General aspects

Acetaldehyde arises as normal by-product of yeast fermentation. Therefore, acetaldehyde was found as a natural constituent in the types of alcoholic beverages investigated. Acetaldehyde levels are dependent on the fermentation conditions, e.g. temperature, O_2 levels, pH, SO_2 levels, and yeast nutrient availability (Ebeler and Spaulding, 1998). While sugar is the primary substrate of acetaldehyde formation, metabolism of amino acids such as alanine, or oxidation of ethanol also contributes to the formation of this compound (Liu and Pilone, 2000). There are large species and strain differences in acetaldehyde production by yeasts; for instance, 0.5–286 mg/l for *Saccharomyces cerevisiae* and 9.5–66 mg/l for *Kloeckera apiculata* (Liu and Pilone, 2000). These influences lead to the fact that the levels of acetaldehyde in alcoholic beverages vary considerably.

Miyake and Shibamoto (1993) found in a very small sample collective, that the acetaldehyde content in alcoholic beverages tends to be roughly equivalent to the ethanol content, meaning that beer contained the least amount of acetaldehyde (5–12 ppm) compared to wine (33–66 ppm) and whisky (25–102 ppm). Linderborg et al. (2008) confirmed this observation and found a significant positive correlation ($r = 0.748$, $n = 49$) between ethanol and acetaldehyde. Our results do not confirm this correlation in general. There are alcoholic beverages with low alcoholic strengths (e.g. beer, wine) that have higher acetaldehyde contents than high-proof spirits (e.g. vodka). Fortified wines with an alcoholic strength between wine and spirits show the highest acetaldehyde concentration. This group of beverages was neither regarded by Miyake and Shibamoto (1993) nor Linderborg et al. (2008), so this discrepancy can be easily explained.

If the acetaldehyde concentrations are calculated for a 'standard drink' of each beverage (Turner, 1990), it appears that the major exposure would derive from wine (especially fortified wine) and to a lesser degree from beer and spirits (Fig. 3). However, spirits could lead to higher local amounts of acetaldehyde in the saliva than wine and beer, even if the absolute ingestion is lower (Table 2). The most problematic group appears to be fortified wine, which has the highest concentration in the beverage and in a standard drink, as well as the highest concentration increase in the saliva.

4.2. Beer

It has been shown that microbiological contamination as well as aeration of the worts are important factors which can result in a higher content of acetaldehyde in beer (Yi and Jingzhang, 2002). Normally, during fermentation acetaldehyde is reduced to ethanol

but it can be oxidized to acetic acid, which is the major volatile acid in beer (Briggs et al., 2004).

In general, acetaldehyde appears to be the least problematic in beer (mean: 9 mg/l). The concentration in beer was generally the lowest of all the product groups. However, it should be noted, that most of the products in our study were manufactured according to the German beer purity law (only water, barley malt, hops and yeast are allowed as raw materials; the use of additives or adjuncts is forbidden). Therefore, further research about acetaldehyde in beer from other raw materials and from other parts of the world is needed.

4.3. Wine

Acetaldehyde is the major aldehyde found in wine. It often constitutes more than 90% of the aldehyde content of wine. Acetaldehyde is one of the early metabolic by-products of fermentation. As fermentation approaches completion, acetaldehyde is transported back into yeast cells and reduced to ethanol. Thus, the acetaldehyde content usually falls to a low level by the end of fermentation (Jackson, 2000). Acetaldehyde formation can be reduced by selecting an appropriate yeast strain and preventing oxidation during vinification (Romano et al., 1994). However, after alcoholic fermentation and removal of the yeast, few alternatives for the reduction of acetaldehyde remain (Osborne et al., 2006).

There is considerable variation in the amount of acetaldehyde present in wines because of increased fermentation temperatures, aeration, higher pH and lack of pantothenate and thiamine resulting in higher acetaldehyde (Wucherpfennig and Semmler, 1972a, 1972b). Acetaldehyde concentration decreased in a majority of red wines during conservation in commercial cellars. Increases were attributed to abnormal conditions of wine exposure to air (Somers and Wescombe, 1987).

It is recommended practice for all table wine types that a positive level of free SO_2 is maintained to ensure the fixation of acetaldehyde with favourable influence on varietal flavour (Somers and Wescombe, 1987). Efficient acetaldehyde-degrading lactic acid bacteria may be applied during malolactic fermentation in wine making. This not only reduces the acetaldehyde content in wine but also reduces the need to bind acetaldehyde with SO_2, which also has health implications (Osborne et al., 2000). Efficient degradation of acetaldehyde was achieved using malolactic fermentation of white wine with *Oenococcus oeni* (Osborne et al., 2006).

Our acetaldehyde results in wine (mean: 34 mg/l) are in good agreement with previous investigations that reported mean contents between 31 and 54 mg/l (Amerine, 1954; Dittrich and Barth, 1984). Only a few products had unusually high acetaldehyde contents (up to 211 mg/l). In such cases, an improvement of manufacturing practices and better hygiene should be demanded from the producers to reduce the acetaldehyde levels.

4.4. Fortified wine

Fortified wines (also called dessert wines or liqueur wines) are wines to which additional alcohol (e.g. brandy) is added. The most common fortified wines are sherry, port, marsala and madeira.

While high levels of acetaldehyde are generally regarded as undesirable in table wines, high concentrations of this volatile compound were considered to be a unique feature of sherry-type wines (Cortes et al., 1998; Liu and Pilone, 2000). Also, port wine owes its essential features of colour and flavour to excess acetaldehyde derived from the brandy used for fortification (Bakker and Timberlake, 1986; Liu and Pilone, 2000).

Considering these facts, the relatively high acetaldehyde content in our sample collective of fortified wines (mean: 118 mg/l) was not unexpected.

The even higher concentration in sherry (mean: 156 mg/l) was also not unexpected as high concentrations of acetaldehyde were described to be typical of aged sherry wines. The acetaldehyde is produced during the biological aging process by 'flor' film yeasts. Depending on the yeast strain, an initial acetaldehyde concentration of 85 mg/l was increased to 146–683 mg/l during 250 days of aging (Cortes et al., 1998). The film yeasts are especially selected for their ability to produce very high acetaldehyde levels (Ebeler and Spaulding, 1998). Besides the yeast strain, aeration during the aging process increases acetaldehyde formation (Begoña Cortes et al., 1999). Experimental productions of sherry wine have shown that acetaldehyde concentrations up to 961 mg/l were possible using certain yeast strains in combination with aeration (Muñoz et al., 2005).

The demand for high acetaldehyde levels for flavour reasons in fortified wines appears to be conflicting with the toxicological properties of acetaldehyde.

4.5. European-style spirits

European-style spirits are defined by law (European Parliament and Council, 2008), however, the regulation provides no limits for acetaldehyde for any of the distilled spirits, which are manufactured by fermentation with retention of the organoleptic properties of their raw materials (e.g. rum, whisky, brandy, fruit spirit).

Only for neutral alcohol (so-called 'ethyl alcohol of agricultural origin'), a limit for acetaldehyde is provided (0.5 g/hl p.a.). Neutral alcohol is for example used for the production of spirits like gin or most liqueurs.

Our results show that European-style spirits (e.g. whisky, brandy) show lower acetaldehyde concentrations than certain spirits from emerging markets (e.g. some Mexican or Asian spirits), so we will discuss the groups separately. We also discuss calvados (a spirit distilled from cider) separately, because calvados was associated in the past with high acetaldehyde levels.

In spirits, acetaldehyde is an undesirable substance due to its unpleasant flavour. During distillation acetaldehyde is enriched in the first fraction, which is generally discarded. A fast colorimetric assay is available to determine the switch-point between the first acetaldehyde-rich fraction (heads) and the desired product fractions of the distillate (Pieper et al., 1987).

During production of fruit spirits acetaldehyde may be formed not only as product of alcoholic fermentation by *Saccharomyces* yeast, but also as a metabolite of microorganisms like lactic acid bacteria or acetic acid bacteria. Therefore, an increased amount of acetaldehyde usually indicates faults during fermentation (Pieper et al., 1987). Using German standard distillation stills most of the acetaldehyde can be separated, however, a complete separation is technically not possible. An average acetaldehyde residue of 12–18 g/hl p.a. (48–72 mg/l) can be found in German fruit spirits according to Pieper et al. (1987). Our results of fruit spirits are in good agreement with these specifications (mean: 21 g/hl p.a.).

Rectification decreases the aldehyde level in distilled beverages to some extent. Because of the low aldehyde content of rectified alcohol used for the production of vodka, the aldehyde content of different vodkas generally is 10 mg/l or less. Relatively larger acetaldehyde amounts can be found in whisky, cognac, brandy, and rum (Nykänen, 1986). This prior view can also be confirmed by our data (Table 1).

4.6. Spirits from emerging countries

In our previous investigation of Mexican spirits, we detected that one kind of spirit (Bacanora) had significantly higher acetaldehyde levels than other groups (Tequila, Mezcal, Sotol), which had

comparable acetaldehyde levels to European-style spirits. This finding may be explained by the fact that most Tequila distilleries employ technological advances, whereas the other types of Mexican alcoholic beverages are manufactured by more rudimentary production methods (Lachenmeier et al., 2006).

Unusually high acetaldehyde levels were also found in spirits from China that were marketed at Chinese restaurants in Germany (mean: 62 g/hl p.a.). These high contents can only be explained by microbiological spoilage leading to acetaldehyde accumulation during fermentation as well as an inadequate separation of the first acetaldehyde-rich fractions during distillation. The analysis of more samples and especially samples from the domestic Chinese market is required to determine if an acetaldehyde problem exists in Chinese spirits.

In contrast, cachaças (sugarcane spirits) from Brazil generally showed comparable acetaldehyde levels to European-style spirits (mean: 13 g/hl p.a.). In a previous report, the average acetaldehyde concentration in 56 cachaça samples was 11 g/hl p.a. with a standard deviation of 4 g/hl p.a. (Nascimento et al., 1997). Another study of sugarcane spirits in Brazil reported an average of 20 ± 13 g/hl p.a. of acetaldehyde (Miranda et al., 2007). In Brazilian cachaça production it was shown that yeast isolated from cachaça samples formed lower acetaldehyde concentrations than a commercial wine yeast, which formed up to 91 g/hl p.a. of acetaldehyde, which exceeded the Brazilian legal acetaldehyde limit of 30 g/hl p.a. (Oliveira et al., 2008). Miranda et al. (2007) also reported that the acetaldehyde limit of 30 g/hl in Brazil was exceeded by 16 out of 94 samples, with 82 g/hl p.a. as the highest level. From these first observations, we can only conclude that spirits from emerging countries might be more susceptible to high acetaldehyde contents because of less-advanced equipment and poorer production hygiene. Possibly, illicitly or home-produced spirits could be more susceptible for acetaldehyde contamination due to the same reasons (Lachenmeier et al., 2007).

Further studies should concentrate on characterizing those products in more details, especially from regions of the world with a higher incidence of upper digestive tract cancer.

4.7. Calvados - a special case

Calvados is a special case because it is the only alcoholic beverage so far, for which an association between the directly ingested acetaldehyde in the beverage and cancer risk was made. Studies researching the accumulation of squamous-cell cancer in Normandie and Bretagne (France) were able to prove a significantly increased risk for cancer of the oesophagus caused by chronic calvados consumption (Launoy et al., 1997, 1998). Consumption of hot calvados appeared to explain about 2/3 of the inter-regional and urban/rural differences in incidence, whereas total alcohol consumption explained less than 1/5 (Launoy et al., 1997). The high concentration of acetaldehyde combined with possible effects of the high temperature at which calvados is consumed could account for the increased risk of calvados-related oesophageal cancer (Linderborg et al., 2008).

There are a number of peculiarities observed during the production of calvados that might lead to unusually high acetaldehyde levels:

First, the cider used for production of calvados may be spoiled by microorganisms producing acetaldehyde. For example, the spoilage called 'framboisé' (cider-sickness) is correlated to the accumulation of high concentrations of acetaldehyde in the medium (150 to 400 mg/l and up to 1000 mg/l while the legal limit in France is 120 mg/l for cider and 100 mg/l in 'cidres bouchés' and 'Pays d'Auge') (Coton and Coton, 2003; République Française, 2000, 1987). The frequency of this spoilage is not constant and can range from 5 to 17% of the annual farm production (Coton and Coton, 2003). The microbiological origin of this spoilage was shown to be caused by the Gram-negative, facultative anaerobic bacterium *Zymomonas mobilis* (Coton and Coton, 2003; Coton et al., 2006). This is the only microorganism known to utilize the Entner-Doudoroff pathway for anaerobic conversion of glucose, fructose or sucrose into ethanol and CO_2, which may lead to a large accumulation of acetaldehyde as by-product (Bauduin et al., 2006; Conway, 1992; Coton et al., 2006; Swings and Deley, 1977). Our results confirm that apple wines contain relatively high amounts of acetaldehyde (mean: 50 mg/l).

Second, the differences might be explained by the French style alembics, which have no trays or appreciable reflux, so that a larger concentration of acetaldehyde may proceed into the heart portion of the distillate (Claus and Berglund, 2005). In a direct comparison between different distillation systems for the production of cider spirits, the use of a double distillation system (Charente type) produced higher levels of acetaldehyde than using a rectification still system, which could be related to the higher distillation time of double distillation (Rodríguez Madrera et al., 2003).

Third, acetaldehyde may form on copper surfaces during distillation as demonstrated in an experimental scale by Dai and Gellman (1993). Formation of acetaldehyde was for example detected during the production of cider spirits on traditional Spanish distillation systems using copper vessels (Rodríguez Madrera et al., 2006). By this formation, the presence of acetaldehyde in the last fractions of the distillate as well as the relatively high levels in the sprits (25–38 g/hl p.a.) in relation to the low concentrations in the ciders (0.9–4.6 mg/l) could be explained (Rodríguez Madrera et al., 2006).

Fourth, an influence of wood type on acetaldehyde was detected in the first phases of the aging process, a greater concentration of acetaldehyde detected with French oak, which could be related to the larger pore size of the staves compared to American oak, permitting the passage of a higher concentration of oxygen (Rodríguez Madrera et al., 2003). The acetaldehyde content in Spanish cider spirits was shown to decrease during aging in American oak barrels. For example, a decrease from 216 mg/l to 187 mg/l (traditional cider distillate) or from 174 mg/l to 147 mg/l (cider distillate from apple juice concentrate) was seen. The acetaldehyde content was higher if traditional production methodology was used (fermentation of freshly-pressed cider apples by wild microflora) in comparison to the manufacture from apple juice concentrate with the use of *Saccharomyces cerevisiae* as starter culture (Mangas et al., 1996).

Neither the general European regulation for cider spirit or cider brandy (European Parliament and Council, 2008), nor the more specific French regulation about calvados (République Française, 1998a) demand maximum limits for acetaldehyde in calvados. However, the French regulation requires that the first fraction of the distillate that is rich in higher alcohols, esters and aldehydes must be separated from the product fractions. For the distillation of calvados with the designations 'Calvados Pays d'Auge' and 'Calvados Domfrontais', the cider used for the distillation is allowed to contain a maximum of 100 mg/l and 200 mg/l of acetaldehyde, respectively (République Française, 2000, 1998b, 1998c).

Linderborg et al. (2008) determined the acetaldehyde concentration of calvados (1780 ± 861 μM, range 451-3928 μM, $n = 25$). Farm-made calvados had the highest mean acetaldehyde concentration of the measured beverages. In our analyses the acetaldehyde concentration of calvados (870 μM ± 334 μM, range 437–1524 μM, $n = 27$) lay in the lower range of the values of Linderborg et al. (2008). This difference can be explained by the fact that we only had a small collective of calvados samples available (products for export, purchased in Germany) and had no farm-made calvados.

4.8. Increase of salivary acetaldehyde concentrations

According to the studies mentioned in the introduction, the potential carcinogenic level of acetaldehyde is approximately 50–100 μM. Linderborg et al. (2008) indicated that the oral and upper digestive tract mucosa is exposed to much higher acetaldehyde concentration after ingestion of calvados (i.e. 20–50 times higher than those considered to be mutagenic). Our study is in full agreement with the results of Linderborg et al. (2008). However, we identified groups of beverages (fortified wine and certain spirits from emerging countries) that pose an even higher risk than calvados as they contain even higher acetaldehyde concentrations.

Our calculations show that the salivary acetaldehyde peak concentration may be increased up to 23,652 μM (Table 2). This is far above the range that was associated with an increased cancer risk by microbiological acetaldehyde production from ethanol in the saliva.

The drinking of alcoholic beverages with such high contents of acetaldehyde might lead to saliva concentrations in the ranges otherwise only found in ALDH2-deficient humans. Therefore, such beverages present a higher cancer risk than beverages with none or only low concentrations of acetaldehyde.

Our results and those of Linderborg et al. (2008) are in contradiction to the previous view that the main source of exposure to acetaldehyde is through the metabolism of alcohol (International Programme on Chemical Safety, 1995). At least for the upper digestive tract, the directly ingested content of acetaldehyde in alcoholic beverages leads to equally high or higher acetaldehyde concentrations as those derived from ethanol metabolism. The acetaldehyde outside ethanol metabolism therefore is an important exposure that has so far been neglected in the carcinogenicity evaluation of alcoholic beverages.

4.9. Suggestion of interventions

4.9.1. Protection against acetaldehyde 'in vivo'

The first animal experiments to identify agents that provide protection against acetaldehyde were conducted in the 1970 s by Sprince et al. (1974, 1975, 1979). L-cysteine and 17 other sulphur compounds were tested in rats. Good protection was obtained with L-cysteine, *N*-acetylcysteine, thiamine-HCl, sodium metabisulfite, L-cysteic acid, and a combination of L-cysteine, thiamine-HCl and L-ascorbic acid. However, the extrapolation of these findings to humans was described to be difficult (Sprince, 1985). Like cysteine, methionine was found to significantly reduce circulating acetaldehyde levels and hepatic acetaldehyde levels in mice and rats (Tabakoff et al., 1989). In mice, cysteine, ascorbate, and lipoic acid caused a statistically significant reduction in acetaldehyde-induced toxicity, while homocysteine afforded only little protection (O'Neill and Rahwan, 1976). More recently, Miyake and Shibamoto (1998) showed *in vitro* that 80–90% of acetaldehyde formation was inhibited by antioxidants like 2″-*O*-glycosylisovitexin or probucol. Animal experiments by Manzardo and Coppi (1991) revealed that L-cysteine, L-ascorbic acid, cysteamine, BHT and propyl gallate and quercetin showed activity against acetaldehyde. Interestingly, wine naturally contains quercetin and other polyphenols (Gorinstein et al., 2000). However, it remains unclear if the concentration of those substances in wine is sufficient to provide protective effects against acetaldehyde.

There also appears to be a general lack of information on whether the results can be transferred to humans. Tabakoff et al. (1989) reported the first results that methionine may lower acetaldehyde in humans ingesting ethanol. A study in humans determined that slow-release buccal tablets of L-cysteine are able to remove two-thirds of acetaldehyde from saliva, which is formed by oral microflora after ethanol intake (Salaspuro et al., 2002). However, the experimental proof that the L-cysteine tablets also bind the indigenous acetaldehyde of the alcoholic beverages and may lead to lower acetaldehyde ingestion is still missing.

The alcohol dehydrogenase (ADH)-inhibitor 4-methylpyrazole is able to reduce salivary acetaldehyde production in ALDH2-deficient humans. However, it did not have any effect in humans with normal ALDH2 (Väkeväinen et al., 2001).

Rota and Poggi (2003) hypothesized that the antimicrobial agent chlorhexidine, formulated as controlled-release chip, and fixed by a dental device (i.e. a modified orthodontic bracket) may be the most rational strategy for reducing acetaldehyde production by microflora.

Clinical trials of such acetaldehyde protecting agents may be warranted. Until then, intervention measures that lead to a reduction of acetaldehyde directly in the alcoholic drinks appear to be more practical.

4.9.2. Reduction of acetaldehyde in the beverages

A feasible way to reduce acetaldehyde in fermented foods is the use of mutant strains of *Saccharomyces cerevisiae*, in which the ADH2 gene is partially disrupted. On a pilot scale, the acetaldehyde content in beer was 2.5 mg/l with the mutant yeast compared to 7.8 mg/l with the unmodified yeast (Wang et al., 2006). However, the use of genetically modified organisms in food production remains controversial.

To avoid the formation of acetaldehyde during the storage of spirits, the absence of air in contact with the spirit is desirable. Hermetically sealed containers should be used and these should be kept full. If this is not possible, a jet of inert gas (N_2) may be used to displace the oxygen. Furthermore, the oxidation is less intense at lower temperatures (Cortés et al., 2003).

The substances used to bind acetaldehyde in the above mentioned *in vivo* experiments are also potentially suitable to bind it in beverages. For example, by adding L-cysteine (1210 mg/l) to beer, the acetaldehyde concentration can be reduced from 146 μM to 10 μM (Suovaniemi et al., 2006). In future research, the advantages of such sulphur compounds as food additives to bind acetaldehyde in beverages should be compared to the traditionally used SO_2.

Furthermore, the addition of sulphur compounds or antioxidants to the beverages in excess might also detoxify the metabolically formed acetaldehyde in the saliva after drinking. Research should be conducted on these substances as a possible means to reduce the carcinogenicity of alcoholic beverages.

4.9.3. Aspects of food policy

Despite the overwhelming proof about the toxicity of acetaldehyde, we have the contradictory situation where acetaldehyde is 'generally recognized as safe' (GRAS) by the US FDA (FDA, 2003) and it is included in the European Union's register of flavouring substances that may be used in or on foodstuffs (European Commission, 1999). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) integrated acetaldehyde in the functional class 'flavouring agent' and commented that there is no safety concern at current levels of intake when used as a flavouring agent (JECFA, 2001). Acetaldehyde was added to food products such as milk products (fruit yoghurt), baked foods, fruit juices, candies, desserts, soft drinks and margarine (Feron et al., 1991).

Already in 1991, Feron et al. concluded that until valid information on the chronic oral toxicity of acetaldehyde, including carcinogenicity is available, acetaldehyde should be considered a potential dietary cancer risk factor for humans. Consequently, Feron et al. (1991) demanded that oral exposure of humans to acetaldehyde should be diminished as far as possible and that this

demand is contradictory to the GRAS status. The research during the last 15 years and our results certainly strengthen this demand.

Recently, the carcinogenic potential of acetaldehyde appears to be proven for its role as metabolite of ethanol and there is a strong evidence that indigenous acetaldehyde in foodstuffs may contribute to the carcinogenicity. Thus, the international and national bodies (JECFA, FDA and EU) should re-consider the status of acetaldehyde. From a public health standpoint, the use of acetaldehyde as flavouring agent should be abolished and the concentration in fermented foods should be reduced as far as possible as a precautionary measure to protect consumers as in the case with other potential human carcinogens (e.g. acrylamide or ethyl carbamate (Lachenmeier, 2007; Lachenmeier et al., 2005b; Wenzl et al., 2007)).

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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