1. Introduction

Acetaldehyde (ethanal, CH$_3$CHO, CAS number 75-07-0) is a volatile compound which belongs to the large family of aldehydes, with a fruity aroma at low levels that turns into a pungent irritating odour at high concentrations (Miyake & Shibamoto, 1993).

Alcoholic beverages contain acetaldehyde in variable amounts. It is also naturally found in many non-alcoholic beverages and in foods (e.g., bread, coffee, ripe fruits) (Uebelacker & Lachenmeier, 2011), as well as in the environment, since it originates from the metabolism of plants. Acetaldehyde is also industrially produced and used as a flavouring (Feron, Til, Vrijer, Woutersen, Cassee, & Rehm, 2009; Linderborg, Salaspuro, & Väkeväinen, 2011). An additional source of exposure can be the release from polyethylene terephthalate (PET) in food packages and beverage bottles (Mutsuga, 2011; Lachenmeier, Kanteres, & Rehm, 2009; Linderborg, Salaspuro, & Väkeväinen, 2011). Another source of exposure can be the release from polyethylene terephthalate (PET) in food packages and beverage bottles (Mutsuga, 2011; Linderborg, Salaspuro, & Väkeväinen, 2011).

Experimental data have shown that the main route of human exposure to acetaldehyde is consumption of alcoholic beverages, followed by cigarette smoking and flavourings used in several foods (Homann, Tillonen, & Salaspuro, 2000; Lachenmeier, Kanteres, & Rehm, 2009; Linderborg, Salaspuro, & Väkeväinen, 2011). Another source of exposure can be the release from polyethylene terephthalate (PET) in food packages and beverage bottles (Mutsuga, 2011; Linderborg, Salaspuro, & Väkeväinen, 2011). Another source of exposure can be the release from polyethylene terephthalate (PET) in food packages and beverage bottles (Mutsuga, 2011; Linderborg, Salaspuro, & Väkeväinen, 2011). Another source of exposure can be the release from polyethylene terephthalate (PET) in food packages and beverage bottles (Mutsuga, 2011; Linderborg, Salaspuro, & Väkeväinen, 2011).

In humans acetaldehyde is a metabolite of ethanol, which is oxidised in the liver to acetaldehyde and then to acetaldehyde dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), respectively (Yokoyama & Omori, 2005). In addition, marked amounts of acetaldehyde are instantly produced in the oral cavity, from ethanol after a single sip of a strong alcoholic beverage, by salivary microbes (Homann, Jousimies-Somer, Jokelainen, Heine, & Salaspuro, 1997; Miyake & Shibamoto, 1993).

Acetaldehyde associated with the consumption of alcoholic beverages has been recently classified as carcinogenic to humans (Group 1) by the International Agency for Research on Cancer (IARC) (Secretan et al., 2009). Cell cultures and animal experiments have shown its carcinogenicity, since it is able to cause point mutations and to form covalent bonds with DNA (Cheng et al., 2003; Fang & Vaca, 1997; Hecht, McIntee, & Wang, 2001; Noori & Hou, 2001; Wang, McIntee, Cheng, Shi, Villalta, & Hecht, 2000). Furthermore, epidemiological studies have suggested that acetaldehyde present in alcoholic drinks or formed endogenously from ethanol oxidation by the oral microflora, is a carcinogen especially in the oral cavity, the oesophagus and the upper digestive tract (IARC, 1999). Thus, acetaldehyde outside of alcohol metabolism could be considered an additional cancer risk (Lachenmeier & Monakhova, 2011; Seitz & Stickel, 2009).

Very limited information is available in the literature about the potential impact of acetaldehyde in foods and beverages on public health. However, Lachenmeier et al. (2009) have developed the
first quantitative risk assessment for acetaldehyde in alcoholic beverages, using the European Food Safety Agency (EFSA) approach for the risk assessment of genotoxic and carcinogenic substances (EFSA, 2005). The authors concluded that alcohol consumption is a direct source of acetaldehyde exposure, which in conjunction with the other sources, results in a cancer risk requiring intervention.

In the present study we measured acetaldehyde in different alcoholic and non-alcoholic beverages consumed in Italy. The aims were: (1) to evaluate acetaldehyde levels in different beverage groups, (2) to investigate which factors contribute to acetaldehyde contamination in beverages, and (3) to assess direct exposure to acetaldehyde from beverages in Italian consumers.

2. Materials and methods

2.1. Sampling

A “convenience” sampling of selected beverages was carried out by the staff of the Environmental Health Sciences Department of IRCCS-Istituto di Ricerche Farmacologiche Mario Negri. One hundred and forty-three samples were taken mainly among drinks in the personnel had at home, with the aim to cover the main categories of alcoholic beverages, normally consumed in Italy. Each sample was collected in a glass vial of 2 ml completely filled, in order to avoid the migration of acetaldehyde into the head space. Wines and beers were taken from bottles just opened, whereas almost all the spirits from bottles already opened. For each beverage selected information, including alcohol by volume, date and site of purchase and date of opening, was collected. Samples were stored at a temperature of 4 °C until analysis.

2.2. Analytical procedure

The analytical procedure used in this work refers to methods reported in previous studies (Wang, O'Reilly, & Pawliszyn, 2004; Ling, Deng, & Zhang, 2008) and based on head-space solid-phase microextraction (SPME) and on isotopic dilution using acetaldehyde-d₄ as internal standard.

Samples preparation consisted of a dilution of variable amounts of alcoholic or non-alcoholic beverages (10–50 μl) in 3 ml of tap water in headspace vials of 10 ml. After addition of 300 ng of the internal standard solution (6 μl of a 50 ng/μl solution), acetaldehyde was then derivatised into a thermally stable and less polar compound by adding 20 μl of o-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBAH). Samples were heated to 60 °C with a hotplate and then extracted using SPME, i.e., exposing a 2-cm triphasic divinylbenzene/carboxen/polydimethylsiloxane fibre (Supelco, Bellefonte, PA) into the headspace for 10 min. After extraction, the fibre was introduced into the gas chromatograph injector, where analytes were thermally desorbed, separated and identified (Müller, Fattore, & Benfenati, 1997).

2.3. Instrumental analysis

Instrumental analysis was performed by gas-chromatography coupled to mass spectrometry (GC–MS) with an Agilent GC5890-MSD5975C-system, in selected ion monitoring (SIM) mode. Acetaldehyde was quantified using the 209 and 213 ions, for native and deuterated internal standard, respectively. A Varian CP-Select 624 CB chromatography column (60 m, 0.32 mm I.D., 1.80 μm film thickness) was used. The GC oven temperature program was: 80 °C for 1 min, rate 15 °C/min to a final temperature of 220 °C, held for 5 min. Helium was used as a carrier gas at a flow-rate of 0.8 ml/min. The injector was operated in splitless mode and temperature was set to 250 °C. Transfer line temperature was maintained at 280 °C. The linearity of the instrumental response was in the range of 0–600 ng/ml with average regression coefficients (ARC) of 0.9995 and limit of detection (LOD) of 0.2 ng/ml. The repeatability, expressed as average coefficient of variation (CV), was 18% (intra-day) and 13% (inter-day).

2.4. Statistics

Data were evaluated using the software Statistica 6.1 (StatSoft, Inc., Tulsa, OK). Spearman's rank correlation analysis was used to investigate the correlation between alcohol content and acetaldehyde concentration. One-way analysis of variance (ANOVA) and Kruskal–Wallis post-hoc test were used to assess differences in acetaldehyde concentrations across beverage categories. Statistical significance was assumed at probability level <0.05.

2.5. Risk assessment

In order to estimate human exposure to acetaldehyde, data provided by the National Research Institute for Food and Nutrition (INRAN) (Leclercq, Arcella, Piccinelli, Sette, Le Donne, & Turrini, 2009) on consumption of the main beverage categories in the Italian population, considering only consumers, were used. The average daily dose of acetaldehyde was calculated by multiplying concentrations found in beverages for the corresponding daily consumption and divided by the average body weight.

For risk characterisation, we applied the EFSA’s margin of exposure (MOE) approach, which considers possible safety concerns arising from the presence in food of substances which are both genotoxic and carcinogenic (EFSA, 2005). The MOE is defined as the ratio between the dose at which a small but measurable adverse effect is first observed in experimental studies, like the no-observed-adverse-effect level (NOAEL) or the lower confidence limit of the benchmark dose (BMDL) (Crump, 1984), and the level of human exposure to the substance considered. In this study a BMDL of 56 mg/kgBW per day corresponding to a 10% increase in cancer incidence as derived in experimental studies (Lachenmeier et al., 2009), was used for the MOE calculation.

3. Results

3.1. Acetaldehyde levels in beverages

Table 1 shows the median value and range of acetaldehyde concentration levels in alcoholic and non-alcoholic beverages for each beverage category. Beers, non-alcoholic beverages, vodka and gin have lower acetaldehyde content than wines, liqueurs and other spirits. Acetaldehyde levels spanned more than three orders of magnitude, ranging from a minimum of 0.78 to a maximum of 1850 mg/l, measured in a gin and in a grappa sample, respectively. Fig. 1 shows concentrations (mean ± SD) of acetaldehyde calculated for all beverage groups. Highest concentrations were detected in grappa samples (561 ± 539 mg/l), followed by fruit-based liqueurs and spirits (119 ± 140 mg/l), sparkling wines (117 ± 55 mg/l) and rum (106 ± 65 mg/l), whereas the lowest were detected in gin (1.20 ± 0.61 mg/l), fruit smoothies (4.40 ± 3.11 mg/l) and tea (4.95 ± 5.11 mg/l).

The Kruskal–Wallis test showed significant differences of acetaldehyde concentration between the beverage categories analysed. In particular, grappa showed significant differences with beers, herb- and spice-based liqueurs and spirits, gin, and non-alcoholic beverages; beers also showed significant differences, with red, white and sparkling wines and with fruit-based liqueurs and spirits; finally, sparkling wines showed significant differences with
beers, gin and fruit-juices. Within the grappa sample group a large variability was observed; homemade grappa samples showed much higher acetaldehyde concentration than those industrially produced (data not shown).

Table 1
Acetaldehyde concentrations\(^a\) (mg/l) in alcoholic and non-alcoholic beverages.

<table>
<thead>
<tr>
<th>Beverage category</th>
<th>Number of samples</th>
<th>Median</th>
<th>Min–Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wines</td>
<td>60</td>
<td>68.0</td>
<td>18.1–477</td>
</tr>
<tr>
<td>Red wine</td>
<td>21</td>
<td>55.8</td>
<td>18.1–477</td>
</tr>
<tr>
<td>White wine</td>
<td>21</td>
<td>67.0</td>
<td>24.7–171</td>
</tr>
<tr>
<td>Rosé wine</td>
<td>3</td>
<td>81.7</td>
<td>71.9–110</td>
</tr>
<tr>
<td>Sparkling wine and champagne</td>
<td>15</td>
<td>123</td>
<td>34.8–254</td>
</tr>
<tr>
<td>Beers</td>
<td>12</td>
<td>8.46</td>
<td>3.62–15.1</td>
</tr>
<tr>
<td>Liqueurs and spirits</td>
<td>61</td>
<td>42.8</td>
<td>0.78–1850</td>
</tr>
<tr>
<td>Fruit-based liqueur and spirit</td>
<td>17</td>
<td>62.3</td>
<td>5.23–483</td>
</tr>
<tr>
<td>Herb- and spice-based liqueur and spirit</td>
<td>11</td>
<td>28.1</td>
<td>2.46–206</td>
</tr>
<tr>
<td>Fortified wine</td>
<td>7</td>
<td>25.8</td>
<td>1.51–127</td>
</tr>
<tr>
<td>Vodka</td>
<td>3</td>
<td>2.11</td>
<td>1.21–38.0</td>
</tr>
<tr>
<td>Gin</td>
<td>3</td>
<td>0.91</td>
<td>0.78–1.90</td>
</tr>
<tr>
<td>Rum</td>
<td>3</td>
<td>137</td>
<td>31.0–150</td>
</tr>
<tr>
<td>Whiskey</td>
<td>3</td>
<td>76.9</td>
<td>11.2–111</td>
</tr>
<tr>
<td>Grappa</td>
<td>13</td>
<td>499</td>
<td>23.4–1850</td>
</tr>
<tr>
<td>Pure alcohol</td>
<td>1</td>
<td>2.45</td>
<td></td>
</tr>
<tr>
<td>Non-alcoholic beverages</td>
<td>10</td>
<td>2.80</td>
<td>0.81–19.1</td>
</tr>
<tr>
<td>Fruit smoothie</td>
<td>3</td>
<td>2.96</td>
<td>2.25–7.95</td>
</tr>
<tr>
<td>Fruit juice</td>
<td>4</td>
<td>4.59</td>
<td>0.81–19.1</td>
</tr>
<tr>
<td>Tea</td>
<td>3</td>
<td>2.64</td>
<td>1.41–10.8</td>
</tr>
</tbody>
</table>

\(^a\) Values rounded off to a maximum of three figures and two decimals.

Fig. 1. Acetaldehyde concentrations (mean ± SD) in alcoholic and non-alcoholic beverages.
Significant differences (Kruskal–Wallis test) were also found between alcoholic beverages made from fruit, alcoholic beverages made from starting material other than fruits (herbs, spices or tubers) and non-alcoholic beverages. Highest concentrations were found in fruit-based alcoholic drinks, followed by spirits without fruit, and non-alcoholic beverages (Fig. 2).

Finally, the Spearman’s rank correlation analysis showed there was not a statistically significant correlation between alcohol content and acetaldehyde concentration. However, when non-alcoholic samples (i.e., fruit juices, cold tea, etc.) were included in the analysis, the correlation was significant ($r = 0.24; p > 0.05$).

### 3.2 Risk assessment

Italian population exposure to acetaldehyde through different alcoholic and non-alcoholic beverages consumption and MOE values are reported in Table 2.

Exposure assessment was carried out for wines, beers and fruit juices, on the basis of consumption data available for mean and high Italian consumers, provided by INRAN (Leclercq et al., 2009). Highest exposure to acetaldehyde, expressed in mg/kgBW per day, were found for mean consumers of wines (0.15 mg/kgBW per day), followed by beers (0.02 mg/kgBW per day) and fruit juices (0.01 mg/kgBW per day), and also for high consumers the highest exposure was for wines (0.81 mg/kgBW per day), followed by beers (0.09 mg/kgBW per day) and fruit juices (0.05 mg/kgBW per day).

MOE results (Table 2) showed the lowest values for mean consumers of wines (368), followed by beers (2614) and fruit juices (7693); for high consumers the lowest value was again for wines (69) followed by beers (654) and fruit juices (1099).

### 4. Discussion

Acetaldehyde content, detected in 143 alcoholic and non-alcoholic beverages consumed in Italy, spans a wide range of concentrations. Highest concentrations were measured in grappa samples, followed by fruit-based liqueurs and spirits, wines and some other spirits, whereas the lowest ones were in gin, vodka, beers and non-alcoholic beverages. In general, fruit-based alcoholic beverages had higher levels of acetaldehyde than the other spirits or fruit-based non-alcoholic drinks. Our results are consistent with data from previous investigations. Lachenmeier and Sohnius (2008) collected a large number of beverages worldwide, measuring acetaldehyde concentrations in the range of 0–1159 mg/l. They found the highest concentrations in fortified wines, followed by spirits, wines and beers, and the lowest in vodka. Furthermore, their study confirmed that fruit-based spirits have higher content of this compound than grain-based spirits. Similarly, Linderborg, Joly, Visapää, and Salaspuro (2008) analyzed several European alcoholic drinks; French Calvados, a distillate produced from apples, contained a higher level of acetaldehyde than other alcoholic beverages.

Acetaldehyde naturally occurs in the investigated beverages, as it is produced by different strains of yeast and bacteria during fermentation. In alcoholic beverages acetaldehyde is an intermediate of alcoholic fermentation by Saccharomyces cerevisiae. In wine, acetaldehyde is also formed by acetic acid bacteria (Liu & Pilone, 2000). Thus, high concentrations of acetaldehyde in fruit-based in comparison to non fruit-/cereal-based alcoholic beverages, as well as the presence of acetaldehyde in non-alcoholic fruit-based beverages, is probably due to the occurrence of this additional pathway, where acetaldehyde is formed as an intermediate of the oxidation of ethanol to acetic acid.

Results of Spearman’s rank correlation analysis show that alcoholic content is not significantly positively correlated to acetaldehyde, contrary to results reported in other investigations (Linderborg et al., 2008; Miyake & Shibamoto, 1993). However, such correlation becomes significant when non-alcoholic samples are included. Thus, from these results it is evident that other factors, in addition to alcoholic fermentation, cause acetaldehyde contamination, since beverages with low alcoholic content (e.g., beers and wines), have higher acetaldehyde levels than high-proof spirits (e.g., vodka and gin) in which, in addition to the different fermentative substrate, the distillation process can also influence the acetaldehyde content.

Few studies (Cortés, Rodríguez, Salgado, & Domínguez, 2011; López Vázquez, Bollaín, Berstsch, & Orriols, 2010) have reported acetaldehyde concentrations in grappa samples. Grappa is a typical Italian spirit made from direct marc steam distillation or distillation after adding water or wine lees (Da Porto, 1998). Results of

![Fig. 2. Acetaldehyde concentrations in alcoholic beverages made from fruit, alcoholic beverages without fruit and made from herbs, spices or tubers, and non-alcoholic beverages.](image)
the present study show a mean level of acetaldehyde in these alcoholic beverages about one order of magnitude higher than the others, confirming the role of both fruit and alcohol in acetaldehyde contamination. However, the high difference between individual grappa samples (almost two orders of magnitude) suggests the influence of the production method on acetaldehyde formation. Distillation under vacuum conditions, for example, causing a lowering of the boiling point, may retain acetaldehyde and other volatile compounds, in order to obtain fruity and floral aromas. In addition, the hygiene of grapes strongly influences acetaldehyde occurrence (Guillamón & Mas, 2011, chap. 9): grapes may be extremely bacterially contaminated as grappa is made by distilling the skins, pulp, seeds, and stems left over from winemaking after pressing the grapes.

Acetaldehyde has been associated both in animal experiments and epidemiological studies with carcinogenicity, and the MOE represents a measure of the risk associated with acetaldehyde exposure. The MOE value gives an indication of the level of concern. The EFSA Scientific Committee considers a MOE of 10,000 or more, based on animal cancer bioassay data, of low concern from a public health point of view and, as a consequence, of low priority for risk management actions (Benford et al., 2010). Results in the present study indicate a MOE lower than 10,000 for all considered beverages, both for mean and high consumers; in particular, a very low MOE was found for high consumers of wines.

These results show, according to those reported in previous studies (Boffetta et al., 2011; Lachenmeier et al., 2009) that acetaldehyde in beverages, combined with the other sources of exposure mentioned above, is a public health concern. We should take into account, that cancer risk could be higher for humans deficient in ALDH, who have been found to have higher levels of acetaldehyde in their lymphocytes (Matsuda, ALDH, 2006). Genetic epidemiological studies showed that the heterozygous ALDH2*1/2 genotype for ALDH2 gene, the enzyme primarily responsible for the oxidation of acetaldehyde during alcohol metabolism, contributes significantly to the development of oesophageal cancer, due to alcohol consumption, in comparison to homozygous ALDH2*1/*1 genotype, which encodes the active enzyme (Lewis & Smith, 2005).

Limitations of this risk assessment are the restricted sample size of each beverage category and the sampling methodology “convenience” sampling) that may have biased the results. Another important limitation is the rather weak toxicological basis for the BMDL, derived for the male tumour-bearing animals, indicating a maximum acetaldehyde concentration of 100 mg/100 ml of pure alcohol (corresponding to about 400 mg/l of grappa), are probably too high to adequately protect beverage consumers.

In conclusion, humans may be exposed to considerable amounts of acetaldehyde from several sources, as it is widely present in foods and beverages, as well as in the environment. Alcohol consumption is the main route of human exposure to acetaldehyde. Evidence provided in this study, and in previous investigations, strongly suggested the importance of a worldwide screening of acetaldehyde levels in many beverages and foodstuffs. Regulatory measures and consumer guidance are needed, to reduce the acetaldehyde presence in food and drinks to as low as technologically possible, and to implement maximum limits for acetaldehyde in alcoholic beverages, intended especially for the ALDH-deficient population. Currently, the regulatory limits, such as that established for grappa in Trentino Alto Adige (an independent region within the Italian Republic), indicating a maximum acetaldehyde content of 100 mg/100 ml of pure alcohol (corresponding to about 400 mg/l of grappa), are probably too high to adequately protect beverage consumers.

Most importantly, the “Generally Recognised As Safe” (GRAS) status of acetaldehyde, an American Food and Drug Administration (FDA) designation, which allows it to be used as a food additive, should be updated and re-evaluated on the basis of the new evidence.
Acknowledgement

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References

Benford, D., Bolger, P. M., Cartwight, P., Coulet, M., Michael DiNovi, M., Lelbach, J. C., et al. (2010). Application of the Margin of Exposure (MOE) approach to substances in food that are genotoxic and carcinogenic. *Food and Chemical Toxicology*, 48, 52–524.


