Xenobiotics in food II. Health risk of xenobiotics in alcoholic beverages

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Abstract

Alcoholic beverages, spirits in particular, have currently come to the forefront of public interest, namely for the health hazards they pose due to their contamination with extraneous substances. The aim of this study was to identify health risk coming from harmful organic substances, which could be promoted or raised up by the presence of ethylalcohol. According to their origin these compounds are classified into several categories: (1) substances that are natural constituents of the product originating in the fermentation process (e.g. some ketones, 1,1-diethoxyalkanes), (2) compounds which are natural part of product but from the point of health risk considered as undesired (e.g. methanol, ethylcarbamate, thujenes, safrole), (3) noxious compounds entering the product through agricultural technologies (e.g. pesticides) and (4) xenobiotics entering the product at certain phases of its production from the external sources and having no relation to production technology (e.g. halogenated hydrocarbons, aromatic hydrocarbons, paraffines, olefines, phthalates, antioxidants etc.).

1 Introduction

The role of beverages containing alcohol has been a prominent feature of life in many societies. For as long as alcohol has been consumed, and that is millennia, the relationship between drinking and various aspects of health have been explored. Although alcohol has been one of the most studied and researched substances consumed by man, there are few

sources one can consult for an objective review of the current state of scientific knowledge in this very broad area.

Epidemiological evidence shows that the consumption of alcoholic beverages increases the risk of developing cancers of the mouth, pharynx and larynx. But ethanol is not a carcinogen by standard laboratory tests[6]. Animal experiments suggest, however, that, given by mouth, it may act as a cocarcinogen in the production of cancers.

Carcinogen is defined as an agent that has initiating capacity and can induce tumours that seldom or never occur spontaneously. Cocarcinogen is an agent that enhances the carcinogenic process without having initiating capacity.

Wheather the cocarcinogenic effects of different alcoholic beverages depend solely on the presence of ethanol and are unaffected by its concentration or by the presence of other constituents in alcoholic beverages is uncertain.

If ethanol is accepted as having promotional or cocarcinogenic properties, it is conceivable that it would enhance a cancerous response to any direct-acting carcinogens also present in alcoholic beverages. Thus, in principle, such mechanism could reduce, increase or have no aggregate effect on risk.

Aim of this study was pointed out the potential carcinogens present in alcoholic beverages as a trace or occasional contaminats.

2 Materials and methods

2.1 Materials

Distillates. Over 200 samples of alcoholic beverages of those available on Czech market, both imported and local production were analyzed. Among them 40 samples of vodka and over 50 samples of fruit brandies were analyzed for the purpose of this study. Average ethanol concentrations in samples were in the range 40-45 % (v/v).
Reagents. Chromatographic and mass spectrometric standards: safrole, isosafrole, thujone, urethane, 1-bromonaphthalene (internal standard) all from Fluka, Switzerland, perdeuterated methanol from Merck, Germany.
Solvents. Dichloromethane (Merck, Germany), acetone (Lachema, CR), methanol (Penta, CR).

2.2 Methods

Gas chromatography (HP 5890J) coupled with mass spectrometry (Jeol DX-303) was employed for determinations.

Methanol. Direct injection of neat sample spiked with internal standard (acetone). Quantification: ions m/z 31 (methanol) and m/z 43 (acetone). Alternatively, isotope dilution with D_4-methanol. GC: Poraplot Q silica capillary column (Chrompack), initial temperature 120 ^{0}C for 2 min, gradient 15 ^{0}C/min up to 180 ^{0}C, helium flow 1 ml/min, injection 1 μl, split 1 : 20.

Volatiles. Stripping (closed loop apparatus) of diluted water solutions of spirits (1:10), charcoal sorbent, extraction by carbondisulfide. GC:60 m silica capillary column 0.32 mm i.d., 0.25 μm film DB-5 (Mega, Italy), initial temp. 35 ^{0}C for 2 min, then gradients of 5 ^{0}C/min to 80 ^{0}C and 10 ^{0}C/min up to 270 ^{0}C. Helium flow 2 ml/min, 1 μl splitless.

Non-volatiles. Methylenechloride extraction of diluted water solutions (1: 4) of spirits. GC: 30 m silica capillary column 0.25 mm i.d., 0.25 μm PEG (INNOWax, Hewlett-Packard). Initial 35 ^{0}C for 2 min, then gradient 10 ^{0}C/min to 250 ^{0}C.

Phthalates. Direct injection of spirits diluted with ethanol (1: 4), internal standard dipropylphthalate, quantification: ion m/z 149. GC: 20 m silica capillary column 0.32 mm i.d., 0.15 μm SE-52 (Mega, Italy), initial temperature 150 ^{0}C for 1 min, then gradient 20 ^{0}C/min to 310 ^{0}C. Helium flow 1.5 ml/min, 1 μl splitless.

Ethylcarbamate. Direct sample injection, MS in selected ion monitoring mode, quantification: ion m/z 62. GC: 25 m silica capillary column 0.31 mm i.d., 0.30 μm Carbowax 20M (Hewlett-Packard) initial temperature 50 ^{0}C for 1 min, then gradient 10 ^{0}C/min to 200 ^{0}C. Helium flow 1.5 ml/min, 1 μl splitless. Sensitivity 10 μg/l.

Mass spectrometry. Electrone or chemical (methane) ionization, EI 70 eV, resolution 1000 or 5000, range of scanned masses 35-500 (EI), 60-500 (CI), rate 1 scan/sec, HRMS calibration with PFK, multiplier 1 resp. 1.8 kV. Transfer lines 250 ^{0}C.

3 Results and discussion

Vodka is an ideal reference material for tracing xenobiotics as the aroma components are present in very low concentrations and peaks overlapping in HRGC chromatogram usually is not significant. Over ninety different samples of vodka and fruit brandies were analyzed. In all over 160 substances have been identified. Of this number approximatively one third can be regarded as xenobiotics. Identified compounds, according to chemical structure, are divided into following groups.

Paraffins and cycloparaffins

cyclohexane
trimethylpentane
n-heptane
methylcyclohexane
2,3,4-trimethylpentane
2-methyl-3-ethylpentane
isooctane
n-octane
isoundecane
n-undecane
n-dodecane
n-tridecane
n-tetradecane

The origin of the first group, saturated hydrocarbons, both aliphatic and monocyclic, is evidently connected with the contact of product with the technological equipment covered with paint containing hydrocarbon dissolvents. As xenobiotics we hesitate to classify long chain n-alkanes, beginning with n-dodecane which were found, although in trace amounts, in practically all brands of vodka, regardless of their origin. These paraffins may originate in the thin protective lipid layer of the grain.

Aromatic hydrocarbons

benzene
toluene
ethylbenzene
m-,p-xylene
styrene
o-xylene
isopropylbenzene
n-propylbenzene
1-methyl-3-ethylbenzene
1-methyl-2-ethylbenzene
1,3,5-trimethylbenzene
1,2,4-trimethylbenzene
indane or methylstyrene
isobutylbenzene
sec.butylbenzene
1,2,3-trimethylbenzene
1,3-diethylbenzene
1-methyl-3-propylbenzene
n-butylbenzene
1,3-dimethyl-5-ethylbenzene
1,3-dimethyl-4-ethylbenzene
n-hexylbenzene
hexenylbenzene
naphthalene
1-methylnaphthalene
2-methylnaphthalene

A large group of xenobiotics form aromatic hydrocarbons, of which 27 have been found in tested beverages. Their presence, just like that of aliphatic hydrocarbons is explainable by the contact with the paints' dissolvents.

Methanol

The presence of methanol in spirits produced fermentatively is of natural origin, probably derived hydrolyticaly from pectin. Pectin is partial methyl ester of polysaccharide substance present in cell walls of all plant tissues which functions as an intercellular cementing material. There is a direct relationship between the pectin content in the raw material fermented and the methanol content in the alcohol produced.

The values obtained give a certain opportunity to compare the methanol content in various types of alcoholic beverages. Taking into account all the differences in the starting raw materials and processing technologies, in fact the only criterion that is common to all the products is the effort to process the raw material most effectively, that is, to obtain the highest product recovery in a quality acceptable to the customer.

The methanol content in the same type of the product made from the same kind of the raw material does not differ markedly regardless of the region or land of origin. On this basis there was compiled the following table.

Type of ethanol	Methanol content of product [mg/l]
Synthetic	0
Sugar cane	≈ 10
Grain starch	10 – 25
Potato starch	50 – 100
Fruit – grapes	100 – 200
Fruit – apples	≈ 500
Fruit – plums	> 1000

Besides alcoholic beverages there have also been analyzed several samples of pure ethanol, obtained fermentatively as well as synthetically. Ethanol of fermentative origin contains the same substances as were found in vodka, likewise the methanol content corresponds to the material processed. Synthetic ethanol does not contain any fatty acids esters and contains no methanol.

The fact that there has been detected no methanol in 24 samples of distillates analyzed can be explained by either their meticulous rectification and refining at the distillery or that there has been used ethanol that has not been produced through the fermentation process.

Alcoholes

Besides mentioned methanol there were identified a series of other alcoholes, which are natural part of fusel oil: 1-propanol, 3-methyl-1-butanol, 2-methyl-1-butanol, 1-pentanol, 2,3-dimethyl-2-butanol, 4-methyl-2-pentanol, 1-hexanol, 1-octanol, β-linalol, sabinol, nerolidol and diethyleneglycol.

Ethers

1-methoxy-4-(2-propenyl)benzene (= p-allylanisole = estragole)
1-methoxy-4-(1-propenyl)benzene (= anethole)
methoxypropenylbenzene (position of substituents not identified)

1-(1-ethoxyethoxy)pentane
1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane (= eucalyptole)

By the number of species identified not an eminet group, but some of them, esp. estragol, are carcinogenic. The more, some of them were found in high concentration (e.g. anethol content in one sample of vodka exeeded 1 g/l). Into this group belongs safrole and isosafrole, both carcinogens, as well. No one of them were found in vodka and fruit brandies.

Acetales

1,1-diethoxyethane
3,3-diethoxypropene
1,1-diethoxypropane
1,1-diethoxy-3-methylbutane
1,1-diethoxy-2-methylpropane
1,1-diethoxyhexane
1,1-diethoxynonane

Acetales are products of reaction of aldehyde with two molecules of alcoholes. They are present practically in all samples analyzed. Sometimes they are a dominant part (esp. 1,1-diethoxyethane) of organics' content, with concentration on the level of several tenth of micrograms in liter of product. Possible health effect is discussed below.

Aldehydes

Aldehydes are one of the fermentation products. Due to the reactivity of aldehyde group, their concentration diminishes with adulteration, they are transfered by reaction with ethanol to 1,1-diethoxyalkanes and via oxidation to acids and corresponding ethyl esters. Found were: acetaldehyde, propionaldehyde, 3-methylbutyraldehyde, 2-butenal, 3-ethoxypropanal, 2-methyl-2-propenal, 1-hexanal, 1-nonanal, 1-decanal and benzaldehyde.

Aliphatic ketones

4-methyl-3-penten-2-on
4-hydroxy-4methyl-2-pentanon
2,6-dimethyl-2,5-heptadien-4-on

Methylpentenon and hydroxymethylpentanon are known contaminants of technical acetone. These substances were found in significant amounts (70 μg/l) in the three alcoholic beverages tested and in smaller amounts in another three. A potential explanation of their presence is that during fermentation of the raw material there occurred some unwanted acetone fermentation. If this explanation is correct, than the whole batch should have been excluded from the further processing. Structurally closed and probably of the same origin is 4-methyl-2-pentanol.

Other identified ketones are: 5-methyl-2-isopropylcyklohexanon (p-menthan-3-on), 1,3,3-trimethylbicyclo[2.2.1]heptane-2-on (fenchone), methylisopropylcyklohexanon, p-mentha-6,8-diene-2-on (carvone) and trimethylbicycloheptane-2-on (camphore). All these ketones are natural constituents of the product.

Fatty acids

Natural part of fermentation products are fatty acids. Free acids are present in trace concentrations only due to rapid esterification to ethyl esters. Found were 2-ethylhexanoic acid, pellargonic, capric and lauric acids.

Esters of fatty acids

Dominant part (till 90%) of organic compounds present in analyzed samples of distillates are esters, mainly ethyl esters of fatty acids. Due to their aromatic properties they belong to more or less desired part of alcoholic beverages. Identified were: ethyl acetate, ethyl propionate, ethyl 2-methyl propionate, isopropyl acetate, ethyl butyrate, butyl acetate, 2-ethoxyethanol acetate, ethyl 2-methylbutyrate, ethyl isovalerate, 3-methylbutyl acetate, 2-methylbutyl acetate, ethyl valerate, 3-methylbutyl propionate, ethyl 3-methylvalerate, ethyl hexanoate, hexyl acetate, ethyl heptanoate, isoamyl isovalerate, heptyl acetate, methyl octanoate, ethyl octanoate, methyl 2-nonenoate, isopropyl palmitate, ethyl nonanoate, menthol acetate, nerol acetate, ethyl decanoate, methyl 2,4-decadienoate, 3-methylbutyl octanoate, 2-methylbutyl octanoate, ethyl 2,4-decadienoate, ethyl undecanoate, ethyl decadienoate, ethyl dodecadienoate, 3-methylbutyl decanoate, 2-methylbutyl decanoate, ethyl tetradecanoate, 2-methylbutyl dodecanoate, ethyl 9-hexadecenoate, ethyl hexadecanoate.

All those compounds are natural part of products analyzed, with exception of isopropyl palmitate (Isopal) which was found in one sample of Lithuanian vodka.Its origin remains unknown, but similar compounds, e.g. isopropyl myristate are used in cosmetics.

Phthalates and other esters

diethylphthalate	dibutylphthalate
diisobutylphthalate	di-(2-ethylhexyl)phthalate

Among esters attention is constantly being drawn to the phthalic acid esters, especially to dibutyl- and di-(2-ethylhexyl)phthalate. Of the other phthalates that we do not encounter in domestic products, there have been detected diisobutylphthalate and diethylphthalate. The presence of phthalates, which are substances suspected of carcinogenity[7] is explained

either by the contact of the product with the protective paints containing a phthalate component or by leaching of phthalates from the plastics used in the manufacture of the technological equipments in which phthalates are used as plasticizers, such as storage tanks, transport tanks etc.

Into "others" identified esters belong dimethyl succinate, diethyl oxalate, dimethyl glutarate and ethyl benzoate.

Ethyl carbamate

Ethyl carbamate (urethane), the ethyl ester of carbamic acid, the potential human carcinogen is commonly present in almost all fermented drinks.There are several pathways known to formation of ethyl carbamate during the fermentation procedure, depending on the nature of material fermented. For example, in fermentation of stone fruits, cyanogenic glycosides present in seeds (e.g.amygdaline) by treatment with β-glucosidases liberate hydrogen cyanide. Oxidation of HCN leads to formation of cyanate and following reaction with ethanol yields ethyl carbamate. Alternatively reaction of HCN with dicarbonyl compounds (e.g. diacetyl or 2,3-pentadione) gave urethane at room temperature[1]. Urethane formation is dependent on the presence of light. It is concluded, that such reaction of HCN, dicarbonyls and ethanol are the most likely mechanisms of formation uraethane in spirits.

Ethylcarbamate level determined depends of the nature of product analyzed, by vodkas the average value is around 100 μg/l, whilst in plum brandies about ten times higher.

Unclassified

trichloroethylene	2,3-dithiabutane
tetrachloroethylene	diethoxydimethylsilane
dibromochloromethane	hexamethylcyclotrisiloxane

The rest of identified compounds with exception of dithiabutane are secondary contaminants, entering the product most probably in the postfermentation phase of production. They were found individually in the distillates originating in the former Soviet Union countries. The dithiabutane is more common species, originated probably from sulphur containing amino acids during the fermentation.

4 Risk assessment

There is no doubt that alcohol consumption in humans is associated with a liver disease which constitutes a significant public health problem. Even among the heaviest drinkers, however, some remain free of liver disease

throughout their lifetime[4]. This means that alcohol consumption is not a sufficient cause of alcoholic liver disease.

The liver plays a dominant role in the metabolism of ethanol with its main pathway for oxidation being alcohol dehydrogenase activity (ADH). By reaction of ADH, ethanol is transformed to aldehyde, which in turn is rapidly oxidized in the liver to acetate. Acetaldehyde is a very potent and reactive compound, and it has been suggested that it is one of the major factors in the pathogenesis of alcoholic liver disease[6]. However, the true pathogenic role of acetaldehyde in the genesis of alcohol liver injury still remains hypothetical. From that point of view appears a question, what is the most important toxic factor – ethanol, its metabolism, or its metabolites?

Completely overlooked remains till now the other main product of acetaldehyde reactivity, the 1,1-diethoxyethane, formed by reaction of oxidative product of ethanol – acetaldehyde with ethanol itself and its possible influence on human health. These types of compounds (acetales) are frequently present in all distillates.

Some of the alkenylalkoxybenzenes, which are found in alcoholic beverages are carcinogenic itself. Safrole (1-allyl-3,4-methylenedioxy-benzene) was the first alkenylbenzene that was shown to be a carcinogen[2]; it acts as a weak hepatocarcinogen in rats and mice. Estragole (1-allyl-4-methoxybenzene) and methyleugenole (1-allyl-3,4-dimethoxybenzene) have hepatocarcinogenic activities in rats and mice comparable to that of safrole[5].

When polycyclic aromatic hydrocarbons were applied locally to the esophagus of mice, their carcinogenic potential was significantly enhanced when ethanol was used as a solvent[3]. In this experiment ethanol most likely has acted as a tumour promoter by its irritating effects on the tissue. In this study, aromatic compounds, beginning with carcinogenic benzene, are occasionaly found in alcoholic beverages.

Next hazardeous and potential human carcinogen present in foods and alcoholic beverages is ethyl carbamate. Under normal dietary habits excluding alcoholic beverages, the unavoidable daily intake is 10-20 ng/kg b.w. On the basis of the evaluation of all toxicity data and its mode of action a conventional risk assessment of ethyl carbamate indicates that this level represents a negligible lifetime cancer risk (less than 0.0001%)[8] Individual habits may greatly enhance the risk, regular drinking of stone-fruit distillates (20-40 ml/day) would raise the hypothetical tumor risk to near 0.01%. People in general and heavy drinkers in particular, often underreport their intake. Because of this underreporting, the threshold amounts of alcohol consumption associated with certain health risk may in fact be higher than those indicating in the existing studies.

We are in full agreement with opinion[2], that is difficult at this point to assess acurately the human health effects resulting from these (aromatic hydrocarbons, phthalates, ethyl carbamate, acetaldehyde, allylalkoxybenzenes, acetales) and other toxicants in alcoholic beverages. However, cumulative exposure to a large number of compounds over an extended period of time could potentially represent a health hazard. An understanding of the mechanism of toxicity should assist in identifying potential toxicants in foods and evaluating risk associated with exposure to them.

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