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Short Communication

ESTIMATION OF COPPER INTAKE IN MODERATE WINE CONSUMERS IN CROATIA

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To estimate Cu exposure level from wine consumption and to assess possible health risk for moderate wine consumers, wine samples were collected from different wine-growing areas of Croatia. Median concentrations were 180 μg L^{-1}, range (76 to 292) μg L^{-1}, in commercial wines and 258 μg L^{-1}, range (115 to 7600) μg L^{-1}, in homemade wines ($P>0.05$). Maximum permitted level of 1000 μg L^{-1} was exceeded in three homemade wines. However, daily intake of Cu from wine (in the range from 0.02 mg d^{-1} to 1.52 mg d^{-1}) estimated from Cu concentration in all wine samples is lower than the tolerable upper intake level of 5 mg d^{-1} proposed by the EU Scientific Committee on Food and does not present a risk to moderate wine consumers.

KEY WORDS: *commercial wines, dietary intake, homemade wines, risk assessment*

The chemical composition of wine is very complex and contributes to the quality of wine. Metals in wine also affect its organoleptic characteristics. Copper (Cu), iron (Fe), and manganese (Mn) often form stable complexes with amino acids and polyphenols, which occur during wine maturation and storage. These complexes determine ageing characteristics, final aroma, taste, and even the colour of wine (1).

Concentration of metals in wine, including Cu, can depend on the contribution from soil on which vines are grown and reach wine through grapes, pesticide treatment used in wine-growing, various steps of the production cycle, and wine processing equipment, conservation, and bottling (1, 2). The concentration of Cu in wine may be lower than in grapes due to a formation of insoluble Cu sulphides during fermentation, which sediment and are removed with yeasts and lees (3, 4).

Intensive and long-term use of Cu-based compounds can have long-term adverse effects on the environment through toxicity to aquatic and soil organisms and can even affect human health. Although Cu is an essential metal for normal activity of many important enzymes, its excessive intake could result in its accumulation and harmful health effects. Symptoms of Cu poisoning include nausea, vomiting, and abdominal and muscle pain, as well as problems with coordination or movement (5). Since Cu deficiency and Cu excess produce adverse effects on organisms, it is important to monitor Cu level in wine, especially in countries with a long wine-producing and wine-consuming tradition such as Croatia. According to the International Organization of Vine and Wine (IOVW) (6), and Croatian legislation (7), the maximum permissible concentration of Cu in wine is 1000 μg L^{-1}.

The aim of this study was to estimate the contribution of wine to dietary Cu intake and possible health risk for moderate wine consumers. Consumption data used in the assessment are based on national average consumption rates and the estimates have been derived for the general population from the analysed wine samples and available peer reviewed literature.

Data were compared with Maximum Permissible Level and Tolerable Upper Intake Level for Cu.

MATERIALS AND METHODS

Sampling and analysis

Ten commercial and fifteen non-commercial, homemade wines were obtained from 13 winemakers from six Croatian wine-growing areas (Dalmatia, Hrvatsko Primorje, Istria, Prigorje, Zagorje and Slavonia). All samples were packed in glass bottles and stored at 4 °C. All reagents were of analytical grade. Working standard solutions were prepared from a 1000 mg L^{-1} Cu standard solution (Merck, Darmstadt, Germany). First, Cu was analysed by flame atomic absorption spectrometry to screen for possible high concentrations, and then, for more accurate determination, by graphite furnace atomic absorption spectrometry (Perkin-Elmer 5100 spectrometer; Perkin-Elmer, Norwalk, CT, USA) with a Zeeman background correction (8). All measurements were performed in two independent replicates. When necessary, wine samples were diluted with Mili-Q water. As matrix modifier, 5 µg of $PdNO_3$ plus 3 µg of $Mg(NO_3)_2 \times 6H_2O$ (both Merck, Darmstadt, Germany) was used. Accuracy was evaluated by standard additions to determine recovery of the spiked sample. Spike recovery was tested with one red and one white commercial Croatian wine, randomly selected between the collected wine samples, because no certified reference material was available. Wine samples were spiked with five concentration levels of Cu, none exceeding 80 µg L^{-1} for either red or white wine samples. The addition of appropriate volume of standard solution of Cu to commercially available Croatian wines resulted in an average recovery of (95±15) % for red, and (100±11) % for white wine. Measurements of ten different replicates of one red and one white wine sample (measured in triplicate) gave relative standard deviations of 1.11 % and 2.58 %, respectively, indicating high within-run precision. The detection limit of 4 µg L^{-1} was calculated as three times the standard deviation of 25 consecutive measurements of the blank sample (3σ). The quantification limit of 13 µg L^{-1} was calculated as ten times the standard deviations of 25 consecutive measurements of the blank sample (10σ).

Statistics

Since the data were not normally distributed, the significance of difference in Cu concentrations between commercial and homemade wines, and between subgroups of white and red wines was calculated using the Mann-Whitney U-test. The test was applied on the total number of samples. Results were considered significant at the 5 % level ($P<0.05$).

RESULTS AND DISCUSSION

Median Cu values were 180 µg L^{-1} (range from 76 µg L^{-1} to 292 µg L^{-1}) in commercial wines and 258 µg L^{-1} (range from 115 µg L^{-1} to 7600 µg L^{-1}) in non-commercial, homemade wines. In all commercial wines and in 10 of 13 homemade wines Cu ranged between 76 µg L^{-1} and 567 µg L^{-1}, which is far below the upper permissible limit of 1000 µg L^{-1} (6, 7). Concentrations above the permitted level were found in three homemade wines, possibly because of too intensive use of Cu-fungicides, too short period between the last application and harvest, or non-compliance to technological norms. No statistically significant difference in Cu levels was found between commercial and homemade wines or between the subgroups of red and white wines.

Our Cu findings for commercial wines are comparable with most reports from other European countries (Table 1). Lower Cu was reported for wines from Argentina (9), and higher Cu for wines from Australia (10), Brazil (11), Czech Republic (12), and Serbia (13, 14).

Before this study, several other authors published data on Cu concentration in Croatian wines (Table 2). Only Banović et al. (15), who measured concentration of Cu, Fe, Zn, and Pb in one Croatian autochthonous sort of red wine produced by various producers over three consecutive seasons, reported Cu level higher than 1000 µg L^{-1} in one wine sample per season. Our results confirm low levels of Cu in Croatian wines.

However, cases of excessive Cu levels suggest that the wine producers should be more aware of possible sources of metals in their wines. Environmental dispersion of fertilizers and pesticides used in viticulture, or contact with copper or bronze materials in winemaking can easily result in elevated concentrations of Cu in wine. Therefore, regular control of metal concentrations in wine during all steps

Table 1 *Concentrations of copper in commercial wine samples from various countries*

Country	Number of samples	γ(Cu) / µg L^{-1}		Reference
		Range	Mean value	
Argentina	-	23 to 28	-	(9)
Portugal	7	50 to 220	100[b]	(26)
Macedonia	4	210 to 250	-	(27)
Bulgaria	2	190 to 250	-	(27)
Spain	10	32 to 330	110	(28)
Slovenia	10	60 to 300	120	(4)
Hungary	35	20 to 642	150±1.34	(29)
Spain	6	28.4 to 490	150[b]	(30)
Italy	16	74.7 to 500	152[b]	(31)
Croatia	25	76 to 292	180	this study
Brazil	29	69 to 1011	197	(11)
Spain (Tenerife)	125	30 to 1190	240	(32)
Germany	150	50 to 394	250	(33)
Italy	4	116.7 to 462	261[b]	(34)
Serbia	5	100 to 460	-	(14)
Turkey	2	160 to 460	-	(27)
Greece	45	200 to 600	-	(35)
Uruguay	47	34 to 650	-	(36)
Australia	24	ND[a] to 1800	330±0.41	(10)
Czech Republic	31	12.8 to 6827	448	(12)
Italy	11	140 to 930	460[b]	(37)
Serbia	8	90 to 2300	580	(13)
Jordan	60	27 to 2600	-	(38)

[a]*Not detected*
[b]*Median value*

of wine production and "corrective treatments suggested by modern oenological techniques" (3) prior to packaging and consumption can prevent excessive metal intake.

Cu is mainly absorbed through the gastrointestinal tract. From 20 % to 60 % of dietary Cu is absorbed, with the rest being excreted in faeces. When Cu homoeostatic control is defective and/or Cu intake is excessive, Cu toxicity may occur. Ingestion of excess Cu is infrequent in humans and is usually a consequence of contamination of beverages (including drinking water) (16). Acute adverse effects of elevated Cu intake are those on the gastrointestinal tract, such as nausea, abdominal pain, vomiting, and diarrhoea. Chronic overexposure to Cu can damage the liver and kidneys. Liver damage has been reported at extremely high Cu intake of >30 mg d^{-1} (5). Recently, several studies have investigated the hypothesis that excessive amounts of Cu in some regions of the brain may trigger and/or speed up a number of neurological disorders including Alzheimer's disease (AD), familial amyotrophic lateral sclerosis (ALS), and Creutzfeldt-Jakob disease (CJD) (17-19). However, the role of Cu in AD, ALS, and CJD is still unclear, and future studies in this area are needed. According to the National Academy of Sciences (20), the tolerable upper intake level (UL) for adults is 10 mg of Cu per day, whereas the EU Scientific Committee on Food (21) has set it to 5 mg d^{-1}. The Recommended Dietary Allowance (RDA) for adult men and women is 0.9 mg d^{-1}.

Based on mean or median and peak Cu concentrations in wine samples from our and other Croatian studies and on reported alcohol annual consumption, we calculated the Cu daily intake by moderate Croatian wine consumers (0.2 L per day) (Table 2). According to the EU legislation (Directive 793/93/EC), "typical" exposure is defined by median (22) and acute exposure by peak Cu concentration

Table 2 *Copper concentrations in Croatian wine across several studies and our assessment of exposure*

Reference	Number of samples	γ(Cu) / μg L^{-1}			Wine consumption / L d^{-1}	"Typical" exposure / mg d^{-1}	Acute exposure / mg d^{-1}
		Min	Max	Mean			
(39)	-	-	-	6	0.2	<0.01	-
(40)	78	10	413	156	0.2	0.03	0.03
(41)	10	32.3[a]	539[a]	-	0.2	-	0.11
(42)	26	48.5	726	-	0.2	-	0.14
This study	25	76	7600[b]	189[c]	0.2	0.04	1.52
(43)	20	90	930	410	0.2	0.08	0.06
(15)	30	212	1230	570	0.2	0.11	0.25

"Typical" exposure is defined by median or mean values.
Acute exposure is defined by the highest concentration.
[a]*Range of mean concentrations*
[b]*The highest value of Cu measured in homemade wine sample*
[c]*Median value*

(23). Average annual consumption of pure alcohol equivalent in Croatia in 2009 was 9.2 L per person (24). For comparison, average annual alcohol consumption in the EU in 2005 was 9.24 L of pure alcohol per person (25). Given an alcohol content of 12 % for wine, this corresponds to an annual wine consumption of approximately 77 L or 0.2 L d^{-1}. Given the median and peak Cu concentrations measured in commercial wine samples from this study and consumption of 0.2 L wine per day, "typical" and acute Cu exposure from wine would be 0.04 mg d^{-1} and 0.06 mg d^{-1}, respectively. Taking into account the highest Cu level measured in a homemade wine sample, acute Cu exposure would be 1.52 mg d^{-1}. These results show that even elevated Cu concentrations found in the three homemade wine samples do not pose a risk to moderate consumers of wine. However, consumption of wine with elevated Cu concentrations can pose a risk to people who drink over 0.5 L d^{-1} of such wine, as they ingest more Cu than the tolerable upper intake level.

Our findings support the need to monitor and control commercial wine Cu levels. The issue at hand however is broad-based education of home winemakers and control of Cu levels in their wines, as they seem to pose a greater risk of exposure.

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Sažetak

PROCJENA UNOSA BAKRA UMJERENOM KONZUMACIJOM VINA

Kako bi se procijenila razina izloženosti bakru prilikom konzumacije vina te utvrdili mogući zdravstveni rizici za umjerene potrošače vina, skupljeni su i ispitani uzorci vina iz različitih vinogradarskih područja Hrvatske. Koncentracije Cu bile su u rasponu od 76 μg L^{-1} do 292 μg L^{-1} (medijan 180 μg L^{-1}) u komercijalnim vinima te od 115 μg L^{-1} do 7.600 μg L^{-1} (medijan 258 μg L^{-1}) u vinima domaće proizvodnje ($P>0,05$). U tri ispitana vina domaće proizvodnje koncentracija Cu bila je iznad najviše dopuštene od 1000 μg L^{-1}. Međutim, izračunani dnevni unos Cu u slučaju konzumacije ispitanih vina (u rasponu od 0,02 mg d^{-1} do 1,52 mg d^{-1}) ne prelazi gornju granicu tolerancije unosa od 5 mg d^{-1} te nije zdravstveni rizik umjerenim potrošačima vina.

KLJUČNE RIJEČI: *dnevni unos, Hrvatska, procjena rizika, vino*

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