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FOOD TECHNOLOGY

Exposure risk to carbonyl compounds and furfuryl alcohol through the consumption of sparkling wines

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ABSTRACT: *The goals of this study were to verify the occurrence of furfuryl alcohol (FA) and carbonyl compounds (acetaldehyde, acrolein, ethyl carbamate (EC), formaldehyde and furfural) in sparkling wines and to evaluate, for the first time, whether the consumption of the samples under study could represent risk to consumers health. These compounds are electrophilic; and therefore, may covalently bind to DNA, which may result in mutagenicity. EC and formaldehyde were present at low levels ($<1\mu g\ L^{-1}$) in all samples. Acetaldehyde, furfural and acrolein were also found in low levels (<1.5, 1.4 and $1.0\mu g\ L^{-1}$, respectively) in 57, 71 and 76% of samples. In the other samples, levels of acetaldehyde, furfural and acrolein ranged from 5.2 to 54.8, 10.5 to 41.0 and 20.3 to $36.7\mu g\ L^{-1}$, respectively. Furfuryl alcohol was also reported in all samples in levels from 10.4 to $33.5\mu g\ L^{-1}$. Acrolein was the only compound reported at levels sufficient to represent risk to health, which occurred in 24% of the samples. A study focused on the origin of acrolein deserves attention, investigating the influence of the concentration of precursors and the role of fermentation in the formation of this aldehyde, besides the evaluation of possible environmental contamination of grapes during cultivation.*
Key words: *risk assessment, sparkling wine consumption, carbonyl compounds, furfuryl alcohol.*

Risco da exposição a compostos carbonílicos e álcool furfurílico através do consumo de espumantes

RESUMO: *Os objetivos deste estudo foram verificar a ocorrência de álcool furfurílico (FA) e compostos carbonílicos (acetaldeído, acroleína, carbamato de etila (CE), formaldeído e furfural) em espumantes e avaliar, pela primeira vez, se o consumo das amostras em estudo poderia representar risco para a saúde do consumidor. Esses compostos são eletrofílicos e, portanto, podem se ligar covalentemente ao DNA, o que pode resultar em mutagenicidade. CE e formaldeído foram encontrados em baixos níveis ($<1\mu g/L$) em todas as amostras. Acetaldeído, furfural e acroleína também foram encontrados em baixos níveis ($<1,5$; 1,4 e $1,0\mu g\ L^{-1}$, respectivamente) em 57, 71 e 76% das amostras. Nas demais amostras, os níveis de acetaldeído, furfural e acroleína variaram de 5,2 a 54,8, 10,5 a 41,0 e 20,3 a $36,7\mu g\ L^{-1}$, respectivamente. O álcool furfurílico também foi encontrado em todas as amostras em níveis de 10,4 a $33,5\mu g\ L^{-1}$. A acroleína foi o único composto encontrado em níveis suficientes para representar risco à saúde, que ocorreu em 24% das amostras. Uma avaliação focada na origem da acroleína merece atenção, investigando a influência da concentração dos precursores e o papel da fermentação na formação do aldeído, além da avaliação da possível contaminação ambiental das uvas durante o cultivo.*
Palavras-chave: *avaliação de risco, consumo de espumante, compostos carbonílicos, álcool furfurílico.*

Furfuryl alcohol (FA) and carbonyl compounds, including acetaldehyde, acrolein, formaldehyde, furfural and ethyl carbamate may form adducts with the DNA due to their electrophilic nature. As consequence, the exposure to furfuryl alcohol (SACHSE et al., 2016), acetaldehyde (ERIKSSON, 2015) and ethyl carbamate (LIU et al., 2017), for example, may increase the risk of cancer in some parts of the human organism, including liver and kidneys. Furthermore, these compounds may be associated with some particular toxic effects, such the relation between formaldehyde and arthritis (OSMAN et al., 2017) and acrolein that play an important role in Parkinson's (AMBAW et al., 2018) and Alzheimer's diseases (BURCHAM, 2017). Furfural toxicity still needs to be better studied, but it is suspected of being a mutagen and might be associated with liver neoplasms (hepatocellular adenomas or carcinomas) (ARTS et al., 2004).

These compounds may be formed from sugars and amino acids, especially during fermentation (RIBÉREAU-GAYON et al., 2006). In addition, acrolein and furfural may be released to the environment and contaminate grapes during

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incomplete combustion processes (petrochemical fuels, wood, cigarette smoking among others) (KENNISON et al., 2007; BURCHAM, 2017). In a previous study, the occurrence of carbonyl compounds (formaldehyde, acetaldehyde, acrolein, furfural and EC) was reported in all stages of vinification, including grapes and the respective wines (FERREIRA et al., 2018). However, toxic levels were reduced throughout Merlot vinification and only the exposure to acrolein revealed represent risk to consumer´s health. In another approach, LAGO et al. (2017) verified that the advancement of ripeness degree and increasing grape maceration time seems to result in higher concentrations of these carbonyl compounds in Syrah wines. Regarding the consumption of these wines, the exposure to acrolein and ethyl carbamate could pose risk to consumer health.

The occurrence of FA and carbonyl compounds (acetaldehyde, acrolein, EC, formaldehyde and furfural) was studied in this research with the objective of verifying, for the first time, the risk of exposure to these compounds through consumption of sparkling wines. Sparkling wines from 21 different wineries of Rio Grande do Sul State, Brazil, were evaluated. Samples were analyzed in triplicate and total acidity, pH and alcohol content were verified according the ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTIS (1995), since these parameters can influence the efficiency of HS-SPME (FERREIRA et al., 2019). The median values of acidity (108meq L^{-1}), pH (3.1) and alcohol (12%) of samples were used in the preparation of the model solution of sparkling wine to perform the calibration curves of toxic compounds. This approach was followed to minimize matrix effects in the analysis quantification.

The estimated daily intake (EDI) and characterization of the exposure risk were obtained following the protocols of the World Health Organization (WHO, 2010),as reported in previous studies (LAGO et al., 2017; FERREIRA et al., 2018; DACHERY et al. 2017). The EDI was expressed in µg kg^{-1} of body weight (BW) per day and calculated as follows:

$$EDI = \frac{[concentration\ of\ toxic\ compound\ (\mu g\ mL^{-1}) \times sparkling\ wine\ consumption\ (mL\ day^{-1})]}{body\ weight\ (kg)}$$

The concentration of toxic compounds was obtained through headspace solid phase microextraction associated with gas chromatography with quadrupole mass spectrometric detection in selected-ion monitoring mode (HS-SPME-GC/qMS-SIM) according previous validated method (FERREIRA et al., 2019).

The consumption of sparkling wine used in the calculation of EDI was 300mL, considering that: (i) the maximum daily alcohol intake should not exceed 30g (equivalent to 39mL of ethanol), as established by the Health Agencies of several countries, including the United States of America, France, Macedonia, New Zealand, Romania, Switzerland, Uruguay and released by the International Alliance for Responsible Drinking (IARD, 2018), and that (ii) the evaluated sparkling wines presented ethanol content between 11.5 and 12.5% (v/v). Furthermore, VÁZQUEZ-AGELL et al. (2007) reported that daily consumption of 300mL of Chardonnay sparkling wine may prevent atherosclerosis due to its polyphenol content.

The Brazilian average weight of 66.5kg, according to Analysis of Personal Food Consumption done by Brazilian government (IBGE, 2011), was used in the EDI calculation.

Acetaldehyde, acrolein, formaldehyde and ethyl carbamate are genotoxic compounds and margin of exposure (MOE) must be used in risk characterization considering the benchmark dose lower confidence limit (BMDL10) as toxicological parameter in the in the calculation:

$$MOE = \frac{BMDL10\ (\mu g\ kg^{-1}\ BW\ day^{-1})}{EDI\ (\mu g\ kg^{-1}\ BW\ day^{-1})}$$

BMDL10 corresponds to the lowest limit of the 95% confidence interval of the dose required to give a 10% increase in the occurrence of a toxic effect compared to the control. BMDL10 values were: 56, 0.36, 0.25 and 28mg kg^{-1} of body weight per day were used for acetaldehyde (LACHENMEIER et al., 2009), acrolein (ATSDR, 2007), EC (SCHLATTER et al., 2010) and formaldehyde (MONAKHOVA et al., 2012), respectively, as already mentioned in a previous study (FERREIRA et al. 2018).

MOE values below 10,000 indicated that the compound poses a potential health risk (WHO, 2010). In contrast, furfural and furfuryl alcohol are non-genotoxic compounds; and therefore, has a safe ingestion parameter set by JECFA (acceptable summative daily intake (ADI) of 500µg kg^{-1} BW) (JECFA, 2000). Risk characterization for these two furan-containing compounds was carried out comparing the EDI with its ADI, where risk may exist if the estimated intake exceeds the ADI.

Carbonyl compounds and furfuryl alcohol were reported in all samples. Table 1 presents the levels, EDI and MOE of these compound found in sparkling wines under study. EC and formaldehyde were not included in table 1, as these compounds were found at low levels in all samples (not quantifiable since these values were between the LOD and LOQ of the method, 0.4 and 1µg L^{-1}, respectively for both compounds), indicating no risk to consumer health. The occurrence of these compounds was reported for the first time in sparkling wines in the present study. The same exposure risk assessment approach adopted for the samples under study was used to verify if the levels of these compounds reported in the literature would pose a risk to consumers' health. In still wines, EC was reported, for example, in samples from China (13.7µg L^{-1}) (ZHANG et al. 2014) and Portugal (54.1µg L^{-1}) (PERESTRELO et al. 2010),which exposure would result in MOE values of 4167 and 1042, respectively; and therefore, with health risk potential according to the threshold of World Health Organization (MOE<10,000) (WHO, 2010). Formaldehyde was found in wines from South Korea and Germany at average levels of 40.9µg L^{-1} (JEONG et al. 2015) and 130µg L^{-1} (JENDRAL et al. 2011), resulting in MOE values of 155,556 and 47,457, respectively; i.e., with no potential for risk to health.

Acetaldehyde, furfural and acrolein were also reported at low levels (concentrations lower than LOQ of the method: 1.5, 1.4 and 1.0µg L^{-1} and higher than LOD: 0.8; 0.5 and 0.7µg/Lin 57, 71 and 76% of samples, respectively), which do not pose a health risk. In the other samples, the levels of acetaldehyde and furfural ranged from 5.2 to 50.5 and 10.5 to 41µg L^{-1}, respectively (Table 1). Levels of these compounds were used for the calculation of the possible exposure, resulting in low EDI ranging from 0.023 to 0.247 and 0.047 to 0.185µg kg^{-1} of BW for acetaldehyde and furfural, respectively. Since

Table 1 - Levels±standard deviation (µg L^{-1}), estimated daily intake (EDI, µg kg^{-1} of body weight) and margin of exposure (MOE, calculated only for genotoxic compounds including acetaldehyde and acrolein) of the toxic compounds reported in the sparkling wines analyzed by HS-SPME-GC/MS-SIM. EC and formaldehyde were not included in the Table, since these compounds were found at levels between the LOD and LOQ of the method (0.4 and 1µg L^{-1}, respectively, for both compounds) in all samples.

	--------------Acetaldehyde--------------			----------------Acrolein--------------			---------Furfural--------		-----Furfuryl alcohol----	
N.[a]	Level	EDI	MOE	Level	EDI	MOE	Level	EDI	Level	EDI
1	<LOQ	<0.007	>8331096	<LOQ	<0.009	>40101	<LOQ	<0.006	15.8±2.0	0.071
2	<LOQ	<0.007	>8331096	<LOQ	<0.009	>40101	<LOQ	<0.006	11.1±0.1	0.050
3	<LOQ	<0.007	>8331096	<LOQ	<0.009	>40101	12.9±0.1	0.058	42±3.9	0.189
4	<LOQ	<0.007	>8331096	<LOQ	<0.009	>40101	<LOQ	<0.006	14.4±0.8	0.065
5	12.4±0.0	0.056	1001075	<LOQ	<0.009	>40101	<LOQ	<0.006	13.7±1.4	0.062
6	50.5±1.7	0.228	245809	33.1±1.7	0.149	2411	15.6±3.3	0.070	15.8±1.7	0.071
7	<LOQ	<0.007	>8331096	<LOQ	<0.009	>40101	<LOQ	<0.006	14.4±1.7	0.065
8	37.9±3.7	0.171	327529	33.4±0.3	0.151	2389	41.0±0.4	0.185	13.9±0.8	0.063
9	<LOQ	<0.007	>8331096	<LOQ	<0.009	>40101	<LOQ	<0.006	11.9±0.7	0.054
10	31.1±0.8	0.140	399143	<LOQ	<0.009	>40101	32.1±0.3	0.145	17.9±0.5	0.081
11	<LOQ	<0.007	>8331096	<LOQ	<0.009	>40101	<LOQ	<0.006	13.4±0.3	0.060
12	<LOQ	<0.007	>8331096	<LOQ	<0.009	>40101	<LOQ	<0.006	16.2±0.6	0.073
13	<LOQ	<0.007	>8331096	<LOQ	<0.009	>40101	<LOQ	<0.006	13.6±0.2	0.061
14	30.5±8.7	0.138	406995	28±1.8	0.126	2850	14.4±3.0	0.065	15.5±0.6	0.070
15	<LOQ	<0.007	>8331096	<LOQ	<0.009	>40101	<LOQ	<0.006	21.9±1.7	0.099
16	5.2±1.6	0.023	2387179	<LOQ	<0.009	>40101	<LOQ	<0.006	17.8±0.3	0.080
17	15.1±4.0	0.068	822075	<LOQ	<0.009	>40101	<LOQ	<0.006	14.2±1.0	0.064
18	54.8±8.9	0.247	226521	36.7±3.0	0.166	2174	10.5±3.4	0.047	20.3±3.4	0.092
19	<LOQ	<0.007	>8331096	20.3±2.4	0.092	3931	<LOQ	<0.006	22.5±2.9	0.102
20	<LOQ	<0.007	>8331096	<LOQ	<0.009	>40101	<LOQ	<0.006	10.4±1.7	0.047
21	10.2±1.6	0.046	1216993	<LOQ	<0.009	>40101	<LOQ	<0.006	33.5±4.0	0.151

a Sample number.

LOQ: limit of quantification of HS-SPME-GC/MS-SIM method for acetaldehyde, furfural and acrolein was 1.5, 1.4 and 1.0µg L^{-1}, respectively, according to the validation procedure previously reported by Ferreira et al. (2019).

acetaldehyde is genotoxic, the MOE was used to characterize the risk, which presented values higher than 10,000 (ranging from 2,387,179 to 226,521, as shown in table 1), indicating that the exposure to this aldehyde does not pose a health risk.

In the case of furfural (non-genotoxic), the EDI has also indicated no health risk, since EDI was lower than the ADI established by JECFA (2000). The same was verified for furfuryl alcohol found at levels from 10.4 to 42μg L^{-1}, which correspond to EDI of 0.047 to 0.189μg kg^{-1} of BW, respectively; and therefore, also lower than the ADI (500μg kg^{-1} of BW). Regarding the occurrence of these compounds reported in literature, only acetaldehyde was previously verified in sparkling wines (WEBBER et al., 2017), which levels was higher (up 60mg L^{-1}, which indicate risk for consumers' health) than those reported in this study.

Levels (20.3 to 36.7μg L^{-1}) and EDI (0.092 to 166μg kg^{-1} of BW) of acrolein found in 24% of the samples under study were sufficient to represent risk to health (MOE values between 3931 and 2174, respectively; i.e, lower than WHO threshold (10,000) (WHO, 2010). This aldehyde was reported for the first time in sparkling wine and has rarely been evaluated in still wines in the literature. In still wines from South African, BAUER et al. (2012) have not detected acrolein and in German wines. KÄCHELE et al. (2015) reported acrolein in still wines at lower levels (0.7μg L^{-1}) than those verified in this study; and therefore without potential to cause health risk (MOE: 120,000 obtained using the same exposure approach adopted for the sparkling wines under study).

Levels of acetaldehyde, EC, formaldehyde, furfural and furfuryl alcohol reported in all sparkling wines do not pose a risk to the health of consumers. However, the occurrence of acrolein deserves attention. This compound was the only whose exposure indicates concern due to the levels detected and its possibility of reacting with the biological nucleophilic targets such as proteins, RNA and DNA, causing cellular dysfunction and/or mutagenicity. In our previous studies, acrolein was found in still wines elaborated using Syrah grapes from São Francisco Valley (LAGO et al., 2017) and Merlot from Campanha Gaúcha (FERREIRA et al., 2018) in sufficient quantities to result risk to human health. This compound was also present in grapes used to winemaking (FERREIRA et al., 2019). Therefore, the environmental contamination of grapes with acrolein due to incomplete combustion processes (petrochemical fuels and wood) or photo oxidation of hydrocarbon found in air may be related the occurrence of this aldehyde in wines.

The role of precursors, fermentation, type of sparkling and storage in the acrolein levels should be elucidated to predict strategies focused on reducing the occurrence of this compound. In addition, it is important to mention that the evaluation of levels of carbonyl compounds and furfuryl alcohol, as well as the monitoring of levels of ochratoxin A and pesticide residues can be important tools for the quality control of wines.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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