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# Influence of ripeness and maceration of the grapes on levels of furan and carbonyl compounds in wine – Simultaneous quantitative determination and assessment of the exposure risk to these compounds

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ABSTRACT

The validated method based on the use of headspace solid phase microextraction (HS-SPME) coupled with the comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometric detection (GC × GC/TOFMS) proved to be appropriate for this first simultaneous quantitative determination of six toxic compounds (formaldehyde, acetaldehyde, ethyl carbamate, furan, furfural and acrolein) found in wines. Acetaldehyde and acrolein coeluted with other wine compounds, which indicated that difficulties could arise if only one-dimensional gas chromatography was used for the determination of these compounds. The advancement of the ripeness degree and increasing the grape maceration time seems to result in higher concentrations of toxic compounds. The exposure to furan, acrolein and ethyl carbamate through wine consumption may pose risks to consumer health, since calculated MOE values were lower than 10,000.



## 1. Introduction

The ripeness degree and grape maceration time are important parameters influencing the chemical profile of wine. The ripeness degree defines the harvest time that must be based on technological and phenolic maturity of grapes. The technological maturity includes soluble solids content of around 20°Brix and titratable acidity of 6–8 g $L^{-1}$ (Ribéreau-Gayon, Dubourdieu, Donéche, & Lonvaud, 2006). Phenolic maturity is related to the quantity and extraction capacity of anthocyanins and tannins obtained from grapes during the maceration. In this stage, the quantity of extractable tannins from grape seed decreases due to the polymerization of these compounds, resulting in lower wine astringency. Furthermore, an increase occurs in the concentration of anthocyanins, which are responsible for the color of grape skins, since the degradation of the cellular walls of skins facilitates the extraction of these compounds during the maceration (Cadot, Caillé, Samson, Barbeau, & Cheynier, 2012).

The enological quality may be satisfactory when grapes with technological and phenolic maturity are harvested, resulting in a wine with the adequate alcoholic degree, "harmonious", pleasant to palate, with balance of astringency and bitterness, and other positive characteristics (Meléndez, Ortiz, Sarabia, Íñiguez, &

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Puras, 2013). Levels considered ideal of soluble solids and acidity in grapes can be achieved in a shorter time with a combination of high temperature and incidence of intense solar radiation on the vine. However, the phenolic maturity may not be achieved under these climatic conditions (Ferrer-Gallego, Hernández-Hierro, Rivas-Gonzalo, & Escribano-Bailón, 2012). In this way, the prolongation of maceration time may resolve problems arising from insufficient phenolic maturity of the grapes at harvest, since the extraction of tannins increases during maceration. The proportion of tannins and anthocyanins affects the stability and color of red wine and the ideal ratio between the concentration of these two groups of phenolic compounds must be equal or higher than 10:1 (tannis: anthocyanins) (Peynaud, 1997).

The effect of ripeness degree and/or maceration time of grapes have been studied in relation to phenolic composition (Ferrer-Gallego et al., 2012), sensory quality (Cadot et al., 2012), physicochemical properties (Meléndez et al., 2013), and volatile profile of wines (Yilmaztekin, Kocabey, & Hayaloglu, 2015). To the best of the authors' knowledge, there is no report about the influence of these parameters on the levels of toxic compounds. Furan and carbonyl compounds represented by acetaldehyde, formaldehyde, ethyl carbamate (EC), furfural and acrolein are toxic compounds that may be formed during winemaking and their quantification in wines has been rarely reported in literature (Jeong et al., 2015; Kächele, Monakhova, Kuballa, & Lachenmeier, 2014; Nóbrega et al., 2015; Perestrelo, Silva, & Câmara, 2015). Acetaldehyde and formaldehyde were found in red wines form South Korea in average levels of 17232.7 and 40.9 $\mu g\,L^{-1}$, respectively (Jeong et al., 2015). The average concentration of EC reported by Nóbrega et al. (2015) in Brazilian wines was 14.6 $\mu g\,L^{-1}$. Fortified wines from Portugal presented furfural in average levels of 1843.1 $\mu g\,L^{-1}$ (Perestrelo et al., 2015). Acrolein was found in German wines at 0.7 $\mu g\,L^{-1}$ (Kächele et al., 2014) and no study has reported quantitative data on furan in wine. Regarding legislation for these compounds, only EC has limits established for wine. Canada (30 $\mu g\,L^{-1}$), Czech Republic (30 $\mu g\,L^{-1}$) and USA (15 $\mu g\,L^{-1}$) are the countries that set maximum levels for this ester in wine. There is no legislation about formaldehyde, however the International Programme on Chemical Safety of World Health Organization (WHO) has established a tolerable concentration of 2600 $\mu g\,L^{-1}$ based on the no-observed-effect level (NOEL) of 260 $mg\,L^{-1}$ for the histopathological effects in the oral and gastric mucosa of rats (IPCS, 2012).

Furan and carbonyl compounds may pose risk to consumer health since they have been related to various diseases. Acetaldehyde exposure may increase the risk of cancer of the upper aerodigestive tract (oral cavity, pharynx, larynx and esophagus), liver, large intestine and breast (Seitz & Stickel, 2010). Formaldehyde may be related to leukemia and nasopharyngeal cancer (Bachand, Mundt, Mundt, & Montgomery, 2010). EC has shown to contribute to the occurrence of tumors in liver, mammary gland (Cui, Wang, Qiu, & Wu, 2016). Furan and furfural may be associated to liver neoplasms (hepatocellular adenomas or carcinomas) (Arts et al., 2004; Dong et al., 2016) and furan has been related to leukemia (Bakhiya & Appel, 2010). Acrolein may play a role in multiple sclerosis, Alzheimer's disease, cardiovascular disease, hepato and nephro-toxicity (Mogue et al., 2015). On the cellular level, the occurrence of the toxic effects of these compounds may be related to DNA and protein adduction, oxidative stress, mitochondrial disruption, membrane damage, endoplasmic reticulum stress, and imune dysfunction (Arts et al., 2004; Bachand et al., 2010; Cui et al., 2016; Dong et al., 2016; Seitz & Stickel, 2010). The International Agency for Research on Cancer (IARC) classifies the acetaldehyde and formaldehyde ingested specifically through alcoholic beverages as carcinogenic to humans (group 1), EC and furan as causing probable (group 2A) and possible (group 2B) carcinogenic effects to humans, respectively, acrolein and furfural are in group 3, in which the IARC needs further study to classify this compound regarding carcinogenic effects (IARC, 2016).

Gas chromatography with mass spectrometric detection has usually been the technique of choice for the determination of these compounds in wine (Kächele et al., 2014; Nóbrega et al., 2015; Paiano et al., 2014). Furthermore, these compounds have been analyzed individually or at best, furan and furfural are analyzed in the same analytical procedure (Perestrelo et al., 2015). The determination of these compounds can be challenging, since they are present in the concentration range of $ng\,L^{-1}$ to $mg\,L^{-1}$ and wine is a complex matrix, in which compounds of different chemical classes are present. Comprehensive two dimensional gas chromatography (GC × GC) offers superior separation capabilities due to high peak capacity, selectivity, sensitivity, structural chromatographic peak organization, when compared to 1D-GC and has already been applied to the investigation of other wine compounds in our previous study (Welke, Manfroi, Zanus, Lazarotto, & Zini, 2012). This is the first report that aims to: (i) develop and validate a method for simultaneous quantification of six toxic compounds (formaldehyde, acetaldehyde, acrolein, furan, EC and furfural) formed during the vinification, using headspace solid phase microextraction (HS-SPME) coupled with GC × GC with time-of-flight mass spectrometric detector (TOFMS); (ii) evaluate the influence of ripeness degree and maceration time of grapes used in winemaking in relation to the levels of these toxic compounds; and (iii) assess the risk of the exposure to these compounds through wine consumption.

## 2. Material and methods

### 2.1. Samples, analytical reagents, and supplies

Syrah wines elaborated by Brazilian Agricultural Research Corporation (Embrapa, from portuguese: Empresa Brasileira de Pesquisa Agropecuária) located in Petrolina, Pernambuco state, Brazil, were evaluated in order to verify the influence of ripeness degree and maceration time of grapes on levels of toxic compounds, in addition to the assessment of the exposure risk to these compounds. Other 11 commercial Syrah wines obtained from different vintages (2010–2013) were also analyzed in relation to the exposure risk. These samples were provided by wineries located in Brazil and Chile (Details on the year and local of production of each sample are in Table S1 of Supplementary Material).

2,2,2-Trifluoroethyl hydrazine (TFEH, Aldrich, Steinheim, Germany) was used as derivatizing agent as suggested by Kim and Shin (2011) to determine aldehydes, including formaldehyde and acetaldehyde, and ketones in water. An aqueous solution of 62,000 $mg\,L^{-1}$ of TFEH was prepared and 100 μL were added to each wine sample before extraction. Sodium chloride (0.3 g NaCl, analytical grade, Nuclear, São Paulo, Brazil) was also added to samples to increase the ionic strength of the solution and consequently improve the extraction of analytes. NaCl was previously dried at 100 °C for 1 h and stored in a desiccator until use.

Standard compounds: formaldehyde, acetaldehyde, EC, acrolein, furan and furfural (analytical purity higher than 98%) were purchased from Fluka (Ronkonkoma, USA) and individual stock solutions (1000 $mg\,L^{-1}$) of each component were prepared in double distilled ethanol. Octanal, 2-furfurylthiol and methyl nonanoate were used as internal standards (IS) and purchased from Sigma (St. Louis, USA). A solution of each IS (1000 $mg\,L^{-1}$) was prepared in double distilled ethanol. A solution (10 $mg\,L^{-1}$) containing the three IS was prepared in double distilled ethanol and 10 μL of this mix was added to each wine sample before HS-SPME. This IS mix (10 μL) was also used in standard solutions intended to elaborate calibration curves. Internal standards were chosen having in