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SM Journal of Food and Nutritional Disorders

Research Article

Changes in Amounts of Possible Carcinogen Acetaldehyde in Wines over Times at Various Temperatures of Storage

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Article Information

Received date: Jun 11, 2018
Accepted date: Jun 28, 2018
Published date: Jun 29, 2018

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Keywords Acetaldehyde; Carcinogen; Gas Chromatography; Organic wines; Storage conditions; White and red wines

Abstract

A method to analyze carcinogenic acetaldehyde levels in wines was developed and its changes inlevels during storage under different conditions were determined. Levels of acetaldehyde were determined in various wine brands and monitored in selected wines stored under various conditions over prolonged periods using a GC/NPD. Levels of acetaldehyde in selected brand wines ranged from 2.22 ± 0.42 to 17.40 ± 4.16 μg/mL in organic white wines, from undetected to 1.60 ± 0.28 μg/mL in organic red wines, from 15.73 ± 1.18 μg/mL to 26.48 ± 4.01 μg/mL in conventional white wines and from 3.01 ± 0.41 to 20.05 ± 1.75 μg/mL in conventional redwines. When selected wines were stored at 6ºC, 28ºC and 40ºC over 120 days, generally, the levels of acetaldehyde increased for up-to 40 days and then decreased. Both white and red organic wines contained less acetaldehyde than conventional wines. In addition, red wines contain lower levels of acetaldehyde than white wines. There is still insufficient data to assess risk of acetaldehyde in wines. Detailed analyses of acetaldehyde in wines stored under different conditions is one avenue in assessing the safety of wine drinking.

Introduction

Wine is one of the most popular alcoholic beverages in the world. The production and consumption of wine is second only to beer among alcoholic beverages. World wine production was estimated to be around 247 million hecto-litters in 2017 [1]. The world trend in wine consumption shows a steady increase. The health-related news of the so-called "French paradox" reported almost three decades ago that wine drinking helped to prevent heart attacks and obesity. Later, it was found that anthocyanidins (red pigments) in red wines possessed potent antioxidant activity preventing the coronary and obesity effects, and counteracting a diet high in fat and calories [2,3]. Despite the health benefits of wine, however, it inevitably contains certain levels of toxic chemicals, including acetaldehyde and formaldehyde [4-6] as well as mycotoxins and pesticide residues [7,8]. Mycotoxins and pesticides are contaminants from the environment and it is possible to set their safety levels. However, acetaldehyde occurs naturally in wine as a metabolic product of ethanol [9]. It also forms from ethanol via yeast fermentation in wine during the production processes and storage [10]. It is extremely difficult to set the safety levels for these naturally occurring toxic chemicals. There are many reports on acetaldehyde associating with various diseases, including cancer [11,12], diabetes [13] and Parkinsonism [14]. At the molecular level, acetaldehyde can bind to DNA or cause point mutations, which can be carcinogenic [15]. The USEPA reported that acetaldehyde is considered to be a probable human carcinogen based on animal studies (Group B2). In addition, erythema, coughing, pulmonary edema, and necrosis could occur at higher exposure levels [16]. These reports indicate that determination of acetaldehyde levels in wines over time under various storage conditions is important in assessing the safety of wine consumption. Trace analysis of low molecular weight carbonyl compounds, including acetaldehyde, is one of the most difficult experimental procedures because they are extremely reactive and miscible in water. However, acetaldehyde was satisfactorily analyzed by a gas chromatograph/nitrogen phosphorous detector (GC/NPD) after it was derivatized to a 2-methylthiazolidine with cysteamine. This derivative has several advantages over other commonly used 2,4- dinitorphenylhydrazine derivatives because the derivatization reaction occurs rapidly with high and stoichiometric yield under mild conditions such as at room temperature and neutral pH [17]. Moreover, 2-methylthiazolidine is stable and reasonably volatile and can be separated perfectly from complex matrices with a GC column, and the excess of the derivatizing reagent, cysteamine, does not interfere with GC analysis. In addition, it contains a nitrogen atom which is highly selective and sensitive to an NPD. This method has been well established and used for many studies [18,19], including for alcoholic beverages [20,21]. In the present study, levels of acetaldehyde were determined in selected wine brands to validate presence of acetaldehyde in wines. The main objective of this research was to investigate the changes

in amounts of acetaldehyde in selected wines, so acetaldehyde levels in selected wines were analyzed over prolonged periods under various conditions using a GC/NPD method.

Materials and Methods

Chemicals and wines

Acetaldehyde, 2,4,5-trimethylthiazole, cysteamine hydrochloride, sodium hydroxide, and sodium sulfate were obtained from Sigma-Aldrich Co. (St. Louis, MO). 2-Methylthiazolidine was bought from Fisher Scientific (Houston, TX). The solid phase extraction cartridge (SampliQ C18 ODS, 1gm/6mL) was purchased from Agilent Technologies, Inc. (Lake Forest, CA). The gas chromatographic internal standard stock solution of 2,4,5-trimethylthiazole was prepared by adding 0.1 g of 2,4,5-trimethylthiazole to 10 mL of ethyl acetate and stored at 6 °C.

A total of 26 brands of wines (5 organic white, 5 organic red, 6 conventional white, 6 conventional red, 1 sparkling, 1 sherry, 1 port, 1 rose) were bought from a local market in Davis, California, USA.

Sample preparation and analysis of acetaldehyde in wines

Twenty mL of each wine sample were added to 20 mL scintillation vials and sealed with screw caps. Samples were stored at 6°C, 28°C and 40°C in the dark. The amount of acetaldehyde in wine was measured at specific intervals of 0, 40, 80, and 120 days. The acetaldehyde in each wine sample was analyzed using a previously reported GC method [17]. Briefully, 2 mL of cysteamine hydrochloride solution (10 mg/mL) was added to 10 mL of each wine sample and then the pH was adjusted to 8.5 with a 2N sodium hydroxide solution. The sample was stirred in a shaking bath at 25°C at 35RPM for 30 mins and subsequently extracted using a solid phase extraction (SPE). The 1000 mg SPE cartridge was washed with ethyl acetate, ethanol, and distilled water in series prior to the addition of a wine sample. After loading 5 mL of wine sample, the SPE was eluted with 5 mL of ethyl acetate. After the eluate was dried over anhydrous sodium sulfate, the ethyl acetate solution was condensed to 2 mL with purging via a purified nitrogen stream then 200 µg of 2,4,5-trimethylthiazole was added as a GC internal standard.

Acetaldehyde in samples was quantified as 2-methylthiazolidine. All samples were prepared in triplicate. Identification of 2-methylthiazolidine was confirmed by comparing its GC retention index and mass spectral fragmentation pattern with those of a standard chemical. The NIST02 mass spectral library (National Institute of Standards and Technology, Gaithersburg, MD, USA) was referred to for further confirmation of identifications.

Instrumental

An Agilent 6890N gas chromatograph equipped with a 30 m × 250 µm × 0.25 µm DB-WAX bonded phase fused silica capillary column (Agilent, Folsom, CA) and a nitrogen phosphorous detector (NPD) was used for quantitative analysis of acetaldehyde as 2-methylthiazolidine. The oven temperature was programmed to rise from 70 to 200°C at 30°C/min and then held for 2 min. The injector and detector temperatures were 200 and 300°C, respectively. The helium carrier gas flow rate was 2.5 mL/min in split-less mode. A typical gas chromatogram obtained in the present study was shown in Figure 1.

An Agilent model 6890 GC interfaced to an Agilent 5973 Network Mass Selective Detector (Foster City, CA, USA) was used for mass spectral identification of 2-methylthiazolidine in a extract from a wine sample. The GC was equipped with a 30 m x 0.25 mm i.d. (d_f = 0.25 µm) DB-WAX bonded phase fused silica capillary column (Agilent, Folsom, CA, USA). The GC conditions were exactly the same as those of the GC/NPD.

Recovery tests

The recovery efficiency of acetaldehyde from selected wines (organic white wine and conventional red wine samples) was examined using 2-methylthiazolidine. Standard acetaldehyde (500 µg) was spiked to each wine sample (10 mL) and acetaldehyde was recovered using exactly the same method as described above. The recovery efficiency was calculated after being adjusted to the amount of acetaldehyde found in the sample wines before it was spiked.

Standard curve preparation for quantitative analysis

A standard curve was prepared by adding known amounts of 2-methylthiazolidine to ethyl acetate solution with 2,4,5-trimethylthiazole as GC Internal Standard (IS). The concentrations were 1, 2, 10, 25, 50, 100, 200, 300 µg/mL. Peak area ratio of 2-methylthiazolidine to IS was used to generate a linear standard curve. The IS concentration in each standard solution was 200 µg/mL. The R2 value of all standard curves were greater than 0.99.

Statistical analysis

Analysis of Variance (ANOVA) was used for data analysis and Tukey's HSD (honest significant difference) test procedure was used to make multiple comparisons with a significance level defined as *p*-value less than 0.05 using R version 3.4.1 (R Foundation for Statistical Computing, Vienna, Austria).

Results and Discussion

In the present study, the recovery efficiency of acetaldehyde from organic white wines and conventional red wines was 51.65 ± 2.98% and 51.42 ± 5.02%, respectively.

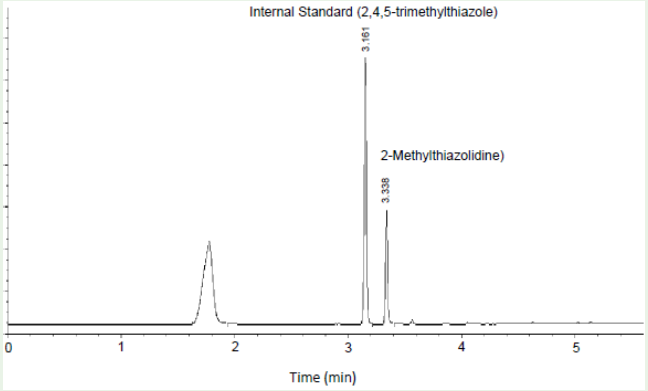

Figure 1: A typical gas chromatogram of organic white wine stored at 28°C for 80 days.

Table 1: Amount acetaldehyde found in commercial wine samples (μg/mL) and their ethanol content (%).

Wine sample	Brand	Amount of acetaldehyde	Ethanol content
Organic white wine	Ia	2.22 ± 0.42	13.5
	Ib	4.48 ± 0.45	12
	Ic	17.40 ± 4.16	13.9
	Id	11.52 ± 0.68	12
	Ie[1]	3.06 ± 0.10	13.5
Organic red wine	IIa	ND[2]	13.3
	IIb	ND	13.2
	IIc	ND	12.5
	IId	ND	13.5
	IIe[1]	1.60 ± 0.28	13.3
Regular white wine	IIIa	26.48 ± 4.01	14.5
	IIIb	21.29 ± 0.30	14.2
	IIIc	23.00 ± 0.20	12.5
	IIId	16.06 ± 7.67	12
	IIIe	23.63 ± 0.41	5
	IIIf[1]	15.73 ± 1.81	13.5
Regular red wine	IVa	20.05 ± 1.75	14.8
	IVb	3.01 ± 0.46	13.9
	IVc	3.65 ± 1.86	13.5
	IVd	3.65 ± 0.50	13.9
	IVe	11.87 ± 2.90	14.5
	IVf[1]	16.11 ± 0.51	14.8
Sparkling wine	V[1]	24.13 ± 1.94	12.5
Sherry	VI[1]	24.15 ± 14.00	17.5
Port wine	VII[1]	19.54 ± 2.25	19
Rose wine	VIII[1]	13.99 ± 1.24	12.5

[1]Used for the time passage experiments

[2]Not detected

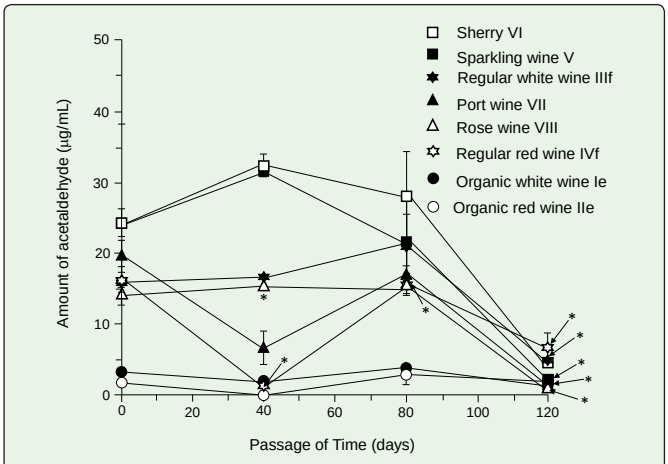

Figure 2: The results of acetaldehyde analysis in wine samples stored at 6ºC over 120 days.

Table 1 shows the amount of acetaldehyde found in selected commercial wine samples along with their ethanol contents. The concentration of acetaldehyde (μg/mL) is presented as mean ± SD (n = 3). Acetaldehyde levels in the samples ranged from not detected (organic red wine, IIa, IIb, IIc, and IId) to 26.48 ± 4.01 μg/mL (conventional white wine IIIa).

White wines contained higher levels of acetaldehyde than red wines in the cases of both organic and conventional wines. This may be due to the presence of antioxidants, in particular anthocyanidins (red pigment), in red wines [22]. Conversely, this may explain why white wines have more acetaldehyde than red wines. Other wines (sparkling, sherry, port, and rose) contained somewhat comparable amounts of acetaldehyde to those of conventional wines.

Figure 2 shows the results of acetaldehyde levels in wines stored at 6ºC, which is a simulated refrigerator temperature. After 40 days stored at 6ºC, acetaldehyde levels in the sample wines ranged from 32.29 ± 1.61 μg/mL (Sherry VI) to not detected (organic red wine IIe). All wine samples except sherry showed reduced levels of acetaldehyde after 40 days at 6ºC. Three patterns were observed in changes of acetaldehyde levels over time. Sherry (VI) and Sparkling wine (V) showed an increase in aldehyde levels for up to 40 days and then the levels decreased. Port wine (VII) and organic white wine (Ie) exhibited reduction of aldehyde levels up to the 40 day mark, but increased again after 80 days to almost the same levels as on day 0. No significant changes in acetaldehyde levels were observed in the other samples. However, after 120 days, the amounts of acetaldehyde in all wine samples decreased significantly. The highest level of acetaldehyde was 6.64 ± 2.90 μg/mL (conventional red wine IVf).

Figure 3 shows the results of acetaldehyde analysis in the wine samples stored at 28ºC, which is a simulated room temperature. Generally, the pattern of Figure 2 is similar to that of Figure 1, except in the case of conventional white wine (IIIf). The amount of acetaldehyde in the conventional white wine sample (IIIf) increased to 33.35 ± 2.02 μg/mL from 15.73 ± 1.81 μg/mL after 40 days. Generally, the amount of acetaldehyde increase or decrease is not significant except in the case of sherry (VI). As in the case of wines stored at 6ºC, the amount of acetaldehyde in all wine samples reduced considerably after 120 days stored at 28ºC.

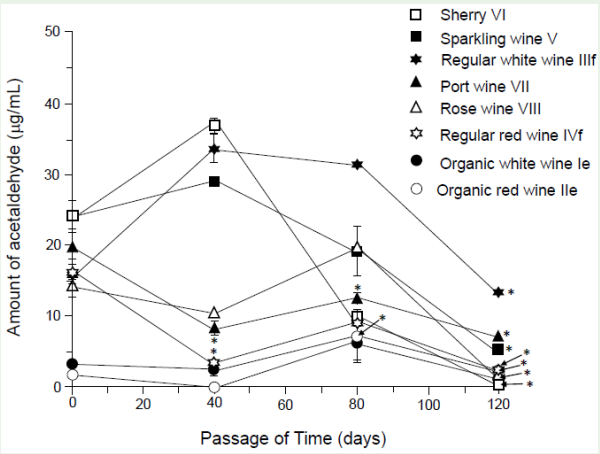

Figure 3: The results of acetaldehyde analysis in wine samples stored at 28ºC over 120 days.

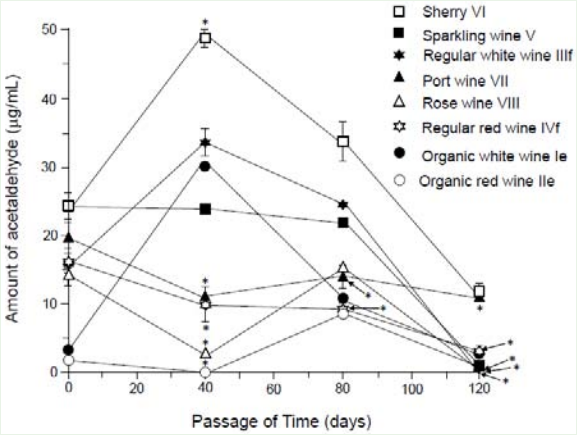

Figure 4: The results of acetaldehyde analysis in wine samples stored at 40°C over 120 days.

Figure 4 shows the results of acetaldehyde analysis in the wine samples stored at 40°C. It is true that wines are stored under low temperature and temperatures for storing wine have never been as high as 40°C. However, refrigerators are not common in homes in some countries, and wines in those places may be stored and consumed at higher temperatures. Also, the temperature may reach 40°C during transportation in tropical areas.

There was a considerable increase in the levels of acetaldehyde in sherry (VI) (48.68 ± 1.30 µg/mL) after 40 days. This level is the highest of all the samples analyzed in the present study. Levels of acetaldehyde in conventional white wine (IIIf) and organic white wine (Ie) increased significantly, indicating that white wines are more susceptible to oxidation. However, it is obvious that the levels of acetaldehyde in all wine samples show a considerable reduction after 120 days passed at 40°C, with the final levels ranging from 0.93 ± 0.13 µg/mL (rose wine VIII) to 11.77 ± 1.14 µg/mL (sherry VI).

During storage, ethanol can be converted to acetaldehyde and further oxidized to acetic acid by nonenzymatic reactions in wine, especially under heat treatment [22]. This may explain why the amount of acetaldehyde generally increased and then reduced during storage. The present study demonstrates that the amount of acetaldehyde present in wines changes during storage under different temperatures. Acetaldehyde may be formed at different stages during the winemaking and storage processes (Figure 5).

The interesting result obtained in the present study is that both white and red organic wines contained less acetaldehyde than conventional wines. It is difficult to rationalize why organic wines contain less acetaldehyde than conventional wines. The one obvious difference between the production method of organic and conventional wine is the use of sulfur dioxide (sulfite or SO_2) in conventional wines. Sulfites have been used as a preservative in wine since the Roman era. But sulfites react readily and reversibly with carbonyl compounds, such as formaldehyde and acetaldehyde, and reduce the amount of carbonyl compounds in wine [23]. One could argue, then, that the addition of sulfites should reduce the amount of aldehyde and consequently, conventional wines would be expected to contain lower levels of acetaldehyde than organic wines that are not treated by sulfites. However, in the present study, the opposite pattern

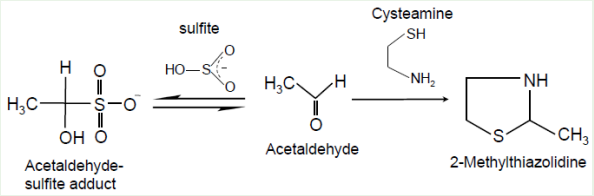

Figure 5: Proposed mechanisms of adduct formation between sulfites and acetaldehyde, followed by cysteamine and acetaldehyde.

was seen. A previous study demonstrated that quantitative analysis of acetaldehyde released from sulfite-adduct reacted with flavonoids in red wines and then subsequently the resulting ethylidene-bridged adduct was successfully analyzed by HPLC [23]. If acetaldehyde trapped with sulfite is released from the adduct, it might form 2-methylthiazolidine with cysteamine. It is proposed that adduct formation between sulfites and acetaldehyde is a reversible reaction as shown in Figure 5 [19]. Therefore, acetaldehyde would be released completely and analyzed as 2-methylthiazolidie in the present study. However, a role of sulfite in acetaldehyde formation in wines is extremely complex [24]. Therefore, further study is required to clarify this phenomenon.

In the present study, analysis of selected commercial wines indicated the presence of acetaldehyde in wines. The results at least demonstrated that organic wines contained less acetaldehyde than conventional wines. Also, red wines contain lower levels of acetaldehyde than white wines. Further studies revealed that the amount of acetaldehyde changed under difference storage conditions (times and temperatures). However, factors leading to the final amount of acetaldehyde present in any particular wine are extremely complicated. Further studies on the investigation of additional possible factors affecting acetaldehyde amounts in wines, such as organic cultivation, production processes and grape varieties with varying geographical sources, are in order. As mentioned above, many reports suggest that acetaldehyde is hazardous to humans at certain levels. However, there is still insufficient data to assess its risk in wines. Detailed analyses of acetaldehyde in wines stored under different conditions is one avenue in assessing the safety of wine drinking.

Acknowledgments

We thank Andrew L Waterhouse for his technical advices.

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