ASSESSMENT OF 5-HYDROXYMETHYLFURFURAL CONTENT IN DRY AND SWEETENED WHITE WINES

EVALUAREA CONŢINUTULUI DE 5-HIDROXIMETILFURFURAL AL UNOR VINURI ALBE SECI ŞI ÎNDULCITE

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Abstract. *5-Hydroxymethylfurfural (HMF) is a water-soluble compound resulting from heating monosaccharides in acidic conditions (e.g. wine pasteurisation), potentially carcinogenic to humans. White wines obtained through classical winemaking technologies and subsequently pasteurised were assessed for their HMF content by UV-vis spectrometry. Different volumes of oversulfited and concentrated musts were added to increase the concentration of sugars in wines (10 to 50 g/L). Samples were subjected to heat treatment (45-100°C) in time intervals correlated with temperature (<120 min). Pasteurised dry wines showed low HMF levels of 1.09-3.14 mg/L. HMF content of traditionally "mulled" wine was the highest in samples sweetened to 100 g/L sugars boiled for 10 minutes (>181mg/L). The HMF content in dry and sweetened white wines was correlated with high sugar content, high acidity, high temperature and a long heating time, normal pasteurisation (75°C, 1-2 min) leading to lower HMF amounts.*
Key words: hydroxymethylfurfural, white wines, pasteurisation, concentrated must, carcinogenic compound

Rezumat. *5-hidroximetilfurfuralul (HMF) este un compus organic hidrosolubil ce rezultă prin încălzirea monozaharidelor în mediu acid (ex. pasteurizarea vinului), având potențial cancerigen. Concentrațiile de HMF ale unor vinuri albe seci pasteurizate au fost determinate prin spectrometrie UV-vis. Pentru a creşte concentrația de zaharuri a vinurilor au fost adăugate volume crescătoare de must suprasulfitat şi must concentrat (10-50 g/L). Probele au fost supuse tratamentului termic (45-100 °C) în intervale de timp corelate cu temperatura (<120 min). Vinurile seci au prezentat valori reduse ale HMF (1,09-3,14 mg/L), în timp ce în vinul „fiert" tradițional îndulcit concentrația de hidroximetilfurfural a fost cea mai ridicată în probele cu 100 g zahăr/L, fiert timp de 10 minute (>198 mg/L). Rezultatele experimentale au indicat o creştere a valorilor HMF odată cu scăderea pH-ului, creşterea concentrațiilor de zahăr şi a temperaturii, pasteurizarea normală (75°C, 1-2 min) conducând la formarea unor concentrații reduse de HMF.*
Cuvinte cheie: hidroximetilfurfural, vinuri albe, pasteurizare, must concentrat, compus cancerigen

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INTRODUCTION

Thermal treatment (with all its variants) is a physical abiotic process of food conservation, aiming to destroy the biodegradable agents and their reserves, and the stabilization of enzymes. Pasteurisation is a procedure applied to young wines intended for short-term consumption, consisting in heating the product at high temperatures for a few minutes. Heating of monosaccharides under acidic conditions gives rise to a high number of furan compounds (Belitz *et al*., 2009).

5-Hydroxymethylfurfural (HMF) is a water-soluble heterocyclic compound, an unsaturated aldehyde, which have in their molecule a furan ring sensitive to oxidation ($C_6H_6O_3$) (Basumallick and Rohrer, 2013). HMF is produced during wine pasteurisation as a result of dehydration of sugars such as fructose and glucose, being an indicator of excessive heat-treatment and wine quality.

Although HMF was not considered harmful, many countries impose restrictions on maximum levels in food and beverages (Basumallick and Rohrer, 2013). The oral HMF LD_{50} for rats was between 1910 and 3100 mg/kg (Ulbricht *et al*., 1984). Recent studies have shown that HMF might be metabolized by humans to potentially carcinogenic compounds (Sachse B. *et al*., 2016).

Concentrated must (CM) is obtained by partial dehydration of fresh grape must, with a legal limit of HMF set at 25 mg/kg (of sugar), while oversulfited must (OM) is obtained by addition of a high amount of sulphurous anhydride in fresh must to prevent fermentation. According to Ţârdea (2007), HMF in wine is formed in amounts between 20 and 100 mg/L, higher contents being present in wines sweetened with concentrated must and in pasteurised wines.

Knowing the quantities of this potentially toxic compound, thermo maceration, pasteurisation or high-temperature bottling of wine can be made more responsible, being aware of the negative effects of a less controlled heating.

MATERIAL AND METHOD

For conducting the study were used the wines of three *Vitis vinifera* L. varieties: Chardonnay, Sauvignon blanc and Fetească regală, from Iaşi vineyard (year 2014), growing in the experimental field of the Research-Development Station for Viticulture and Winemaking Iasi - Romania. Grapes were harvested at technological maturity, destemmed, mechanically crushed and pressed. After wine fermentation (in stainless steel vats), fining was done with bentonite (0.9 g/L). After filtering and SO_2 addition, wine pasteurisation was conducted in the industrial installation (75 °C, 2 min). Calculated volumes of CM and OM were added in wines in order to obtain sugar concentrations of 10, 30 and 50 g/L. Wine samples were heated to 45 °C (60-80-100 min), 75 °C (1-2-3 min) and 100 °C (10-20-30 sec). Traditional “mulled” wine was achieved by adding 50, 75 and 100 g/L commercial sugar in the dry wine (Chardonnay) and boiling (95.50 °C) for 5 and then 10 minutes on laboratory burner.

Physical and chemical features of wines were analysed according to the Compendium of International Methods of Wine and Must Analysis (OIV, 2012).

HMF content of dry and sweet wines was determined using the colorimetric method with barbituric acid (reference method) OIV-MA-AS315-05A (OIV, 2012). HMF aldehyde reacts with barbituric acid (0.5% in distilled water) and para-toluidine (10% in

isopropanol) forming a red-coloured compound that is determined by spectrophotometry at 550 nm. Measurements were carried out using a UV-vis spectrophotometer UV-Mini 1240 series (Shimadzu, Japan) and glass cuvettes with 10 mm optical path. HMF content expressed in mg/L was calculated with the following formula, according to STAS 6182/29-73 (1997):

(1) for samples with a free SO_2 content up to 10 mg/L: HMF=34.2×E×F;

(2) for samples with a free SO_2 content up to 10 mg/L: HMF=57.0×E×F;

where: 34.2 and 57.0 are coefficients calculated based on the calibration curve for correspondence between the HMF content (mg/L) and extinction for sample; E is the maximum value of extinction at 550 nm and F is dilution factor.

The values shown represent the mean of at least two determinations. One-way analysis of variance (ANOVA) test was initiated to investigate significant differences between data (Microsft Excel®). P values lower than 0.05 ($p<0.05$) were significant.

RESULTS AND DISCUSSIONS

Physico-chemical characteristics of analysed wines and musts are presented in Table 1. Chardonnay wine had the highest content of residual sugars (1.72 g/L), while Feteasсă regală wine presented the highest acidity and the lowest pH.

Table 1

Physico-chemical features of analysed samples

Sample	Density (g/cm^3)	Alcohol (vol. %)	Total acidity (g/L)	pH	Volatile acidity (mg/L)	SO_2 (mg/L)		Sugars (g/L)	Not. Red. extract (g/L)
						Free	Total		
Sauvignon blanc	0.9914	12.20	6.26	3.58	0.24	38	110	1.28	16.72
Chardonnay	0.9896	13.70	5.80	3.62	0.25	31	92	1.72	17.68
Feteasсă regală	0.9932	11.50	6.82	3.49	0.22	34	112	1.23	20.67
CM	-	-	8.21	3.01	0.44	21	49	735.00	-
OM	-	-	7.01	3.21	0.38	701	1064	171.40	-

Note: CM - concentrated must; OM - oversulfited must; Not. red. - Not reducing extract.

HMF content of white dry wines pasteurised in industrial installations varied within the range of 1.08 and 1.43 mg/L, being highly correlated with the sugar content of samples (fig. 1). Thus, wines with a higher content of residual sugars showed higher HMF concentrations after industrial pasteurisation.

Also, it was observed an inverse relationship between the content of HMF and the total acidity of wines. Wines with a high acidity (and lower pH) contained higher amounts of HMF ($R^2 = 0.9036$). Kuster and Temmink (1977), showed that HMF formation is favored at pH 2.7-3.9. Previous research indicated that reducing the pH in wine (from 3.35 to 3.05, 2.92 or 2.36) caused a significant increase in HMF production (Amerine and Joslyn, 1970).

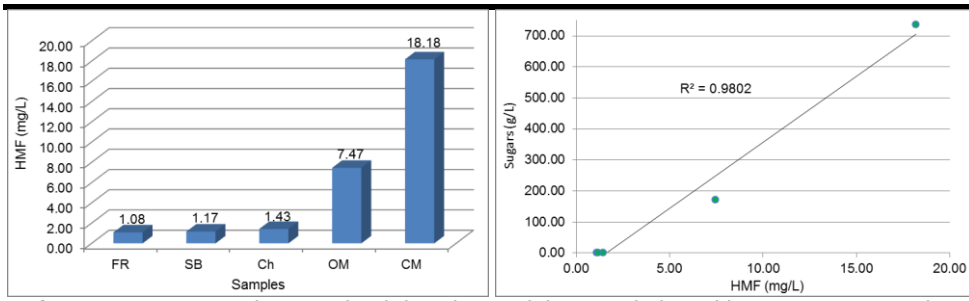

Fig. 1 HMF content of pasteurised dry wine and the correlation with sugar concentration HMF – 5-hydroxymethylfurfural; FR – Fetească regală; SB – Sauvignon blanc; Ch – Chardonnay; OM – oversulfited must; CM – concentrated must.

Heating the dry and sweetened wine samples to 45 °C led to formation of high HMF concentrations, with values increasing up to 58.14 mg/L in the case of wine sweetened with CM to 50 g/L sugars (fig. 2). After 120 minutes at 45 °C, the HMF concentration was 10.3 times higher for wines sweetened with CM to 50 g/L sugar and with about 21.2 times for wines sweetened with OM to 50 g/L.

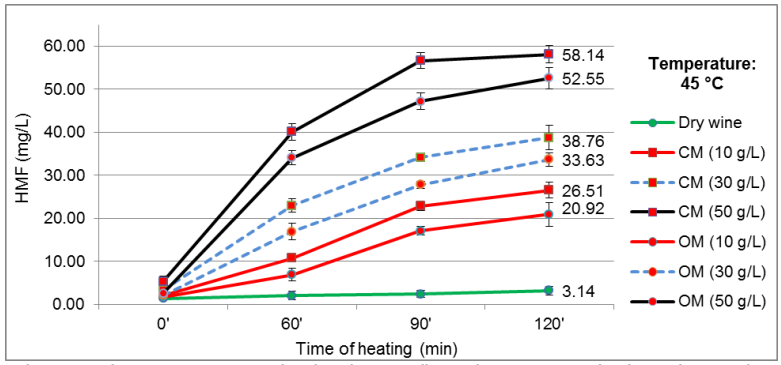

Fig. 2 Changes in HMF content in dry (control) and sweetened wines heated at 45 °C HMF – 5-hydroxymethylfurfural; CM – concentrated must; OM – oversulfited must. Error bars indicates standard deviation (n=2).

Heating the wine samples at 75 °C but for a shorter time, lead to the formation of lower HMF concentrations in comparison to the long exposure at lower temperatures. HMF was synthesized differentially during heating, depending on the amount of sugar in samples. At higher concentration of sugars, HMF increased more in the first minute of heating (fig. 3). The highest HMF amount was recorded in wines sweetened with CM at 50 g/L, due to the existence of higher initial HMF concentration.

Regardless of the sugars concentration, heating the samples to 100 °C for 30 sec resulted in the formation of larger amount of HMF compared with exposure at 75 °C for 3 min, but lower than the heating at 45 °C for a longer time (120 min) (Fig. 4). In dry wine, HMF increases were the lowest but the synthesis dynamic was constant throughout the period of heat exposure.

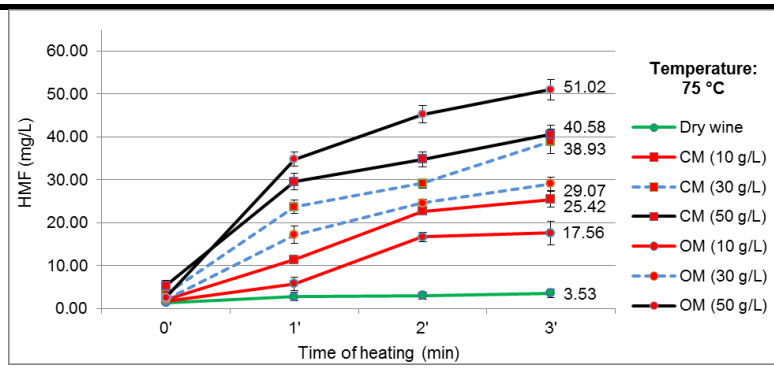

Fig. 3 Changes in HMF content in dry (control) and sweetened wines heated at 75 °C
HMF – 5-hydroxymethylfurfural; CM – concentrated must; OM – oversulfited must. Error bars indicates standard deviation (n=2).

According to Amerine and Joslyn (1970), young white dry wines have about 0.80 to 2.40 mg/L HMF, a slow increase of HMF (2.00 mg/L) occurred when wines of 0.4 to 3.4 g/L reducing sugars were heated at 50 °C for 12 hours, while at 70 °C much higher amounts of HMF were formed (20 mg/L).

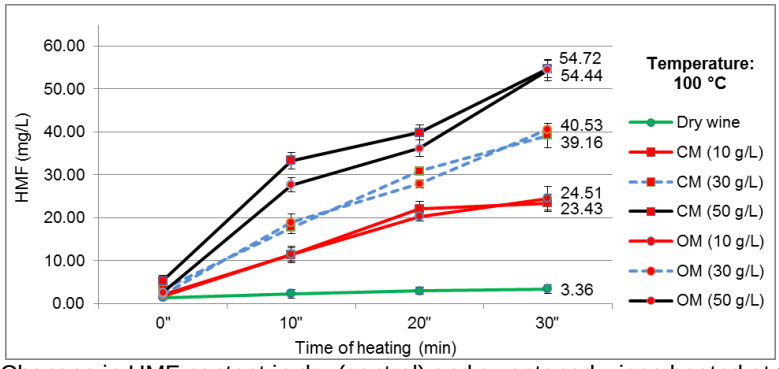

Fig. 4 Changes in HMF content in dry (control) and sweetened wines heated at 100 °C
HMF – 5-hydroxymethylfurfural; CM – concentrated must; OM – oversulfited must. Error bars indicates standard deviation (n=2).

In unsweetened natural wines HMF is found as traces (up to a maximum 2.5 mg/L), while the sweetened wine using concentrated grape must or heated, the HMF content may exceeds 300 mg/L (Ţârdea, 2007).

HMF content of the traditionally prepared sweetened „mulled" wine was very high, increasing with sugar concentration and the heating time. After five minutes of boiling, in dry wine the HMF content was 6.69±1.21 mg/L, while in the wine sweetened with 100 g/L sugar was over 164 mg/L (Fig. 5). Doubling the boiling time, HMF increased statistically insignificant ($p = 0.052$), reaching up to 181.12 mg/L in wines sweetened with commercial sugar to 100 g/L.

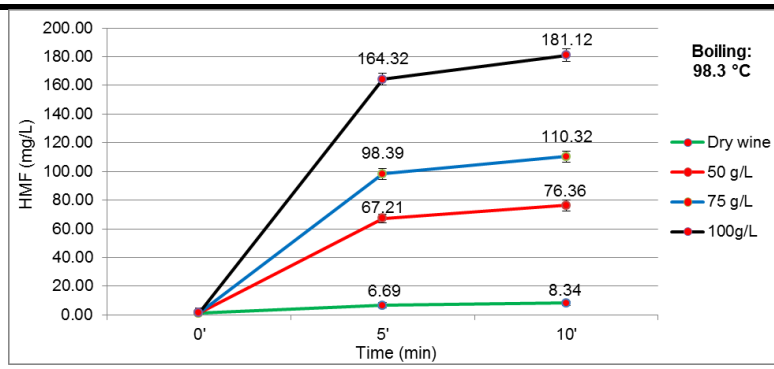

Fig. 5 Changes in HMF content in dry (control) and sweetened mulled (boiled) wines HMF – 5-hydroxymethylfurfural; CM – concentrated must; OM – oversulfited must. Error bars indicates standard deviation (n=2).

CONCLUSIONS

1. The HMF content in dry and sweetened white wines after pasteurisation was low, correlated with high sugar content, high acidity, high temperature and a long heating time.

2. Normal pasteurisation (75 °C, 1-2 min) leads to the formation of lower HMF concentrations comparing to long time pasteurisation at lower temperature.

3. HMF content in mulled wine increased up to 181 mg/L, which arouses concern about food safety of these products.

4. HMF formation must be carefully monitored during pasteurisation, thermo-maceration, high-temperature bottling or storage at higher temperatures, to avoid the accumulation of this potentially carcinogenic compound in wines.

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