

# Determination of 5-Hydroxymethylfurfural in Vinegar Samples by HPLC

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The aim of this study was to establish an overview of the HMF (5-hydroxymethylfurfural) content of different vinegar samples. A reverse-phase HPLC method with diode-array detection without extensive sample cleanup was used. The recoveries of HMF in spiked vinegar were found to be in the range of 95–101%. The detection limit was in the range of 80  $\mu\text{g/L}$ . The sample preparation consisted simply of dilution and filtration of the vinegar samples. The HMF content was analyzed in various kinds of vinegar ( $n = 120$ ) and balsamic vinegar ( $n = 35$ ). Depending on their HMF content the vinegars could be divided into four groups: samples with no, low, medium, and high HMF concentrations. Few vinegar samples investigated contained no HMF. Most of the other samples contained HMF in a low range up to 10 mg/L, whereas sherry vinegar and some apple vinegars had concentrations up to 35 mg/L. Only the balsamic vinegars showed a very high concentration of 300 mg/L to 3.3 g/L of HMF. The highest concentrations were found in traditionally produced balsamic vinegar samples. Depending on their age, up to 5.5 g/kg could be determined.

**Keywords:** 5-Hydroxymethylfurfural (HMF); vinegar; balsamic vinegar; HPLC

## INTRODUCTION

HMF (5-hydroxymethylfurfural) can be formed by heat treatment of food which is rich in sugars. It is the result of the Maillard reaction (nonenzymatic browning) and/or the end product of acid-catalyzed hexose (e.g. fructose, glucose) degradation via the formation of mostly unknown intermediates. HMF is widely spread in food products. It is often used as an index of heat treatment and of deteriorative changes in food such as tomato paste, honey, and fruit juices. In addition, HMF is an indicator of adulteration of food products with acid-converted invert syrups.

Vinegar is produced by microbiological fermentation of ethanol-containing substrates. Depending on the origin of the food matrix, different types, e.g. wine, apple, sherry, or malt vinegar, can be distinguished. Aqueous ethanol (e.g. industrial quality or distilled spirits) can also be fermented, and this gives a colorless spirit vinegar. Another method of production is chemical synthesis which results in a highly concentrated acetic acid solution. This pure acetic acid is diluted with water to 60–80 vol % and used as vinegar essence. A specific procedure is applied for the production of balsamic vinegar. This Italian wine vinegar speciality is known as "Aceto Balsamico di Modena". In the first step, the fresh grape must is concentrated up to half of its original volume by a slow heating process. This sugar-rich solution serves as a substrate for bacteria which ferment the sugar into acetic acid. The traditional method of production requires storage in different wood barrels for up to 25 years. In contrast to this traditionally produced balsamic vinegar, other basamic vinegar is produced by blending the concentrated must with acetic acid, and this mixture is then allowed to ripen for several years in wooden barrels to develop the typical organoleptic characteristics (Bortolotti, 1993).

As all food matrixes used for the production of vinegar contain sugar, formation of HMF during either the

production process or storage is possible. The content of HMF can vary largely in the various food products. Whereas the values for HMF in wine, spirits, and fruit juices were found to be in the range 0–200 mg/L, relatively high concentrations of HMF were observed in prune juices varying from 0 to 1000 mg/L (Van Gorsel et al., 1992). Another possible source of HMF in food products is the addition of caramel solutions.

In recent studies it was concluded that sugar heated under household cooking conditions could act as an initiator and promoter of colon cancer because of the presence of HMF (Bruce et al., 1993; Zhang et al., 1993). The fact that the HMF content in commercial samples is controllable while this is not the case in food prepared in households under individual conditions makes the estimation of HMF intake difficult. Moreover, vinegar is normally used as a condiment, and therefore the contribution to the daily HMF intake should be negligible.

Numerous analytical methods have been developed for the determination of HMF in various food products. Spectrophotometric methods have been used for many years and are often the official method for the determination of HMF in food. However, the original Winkler method involves the use of the toxic compound *p*-toluidine and is rather complicated by uncertainties in the color measurement. This reaction and the reaction with 2-thiobarbituric acid have been used to quantify HMF in honey (Wood, 1993), wine (Malik et al., 1985; Navara et al., 1986; Guerra Hernandez et al., 1988; Montilla Gomez et al., 1988), brandy (Quesada Granados et al., 1992; Duran Meras et al., 1995), grape syrup, and must (Malik et al., 1981). In general, these methods lack specificity because the total amount of furanic aldehydes is determined. Derivative spectrophotometry has been applied for the determination of HMF in citrus fruit juices (Espinosa-Mansilla et al., 1992).

The chromatographic techniques in use are based on gas chromatography (Guerra Hernandez et al., 1988; Rapp et al., 1985), ion exclusion chromatography (Kim and Richardson, 1992), and micellar electrokinetic chromatography (Corradini and Corradini, 1992). Recently, several HPLC methods have been published using UV detection at 280–285 nm. HMF was determined by HPLC in spirits and honey (Jeuring and Koppers, 1980; Vinas et al., 1992), wine (Laszlavik et al., 1995; Chatonnet et al., 1992) and other alcoholic beverages (Frischkorn et al., 1982; Coco et al., 1995), fruit juices (Fuleki and Pelayo, 1993; Grosheny et al., 1995; Rizzolo et al., 1989; Coco et al., 1995; Lee et al., 1986; Ruggieri and Ruggieri, 1982), and coffee (Dauberte et al., 1990).

The aim of this work is to investigate the HMF content so as to give an overview of the actual concentration in different vinegar samples. With the exception of wine vinegar, especially sherry wine vinegar (Garcia-Parrilla et al., 1996), no values for HMF concentrations in other vinegar samples have been reported in the literature. As previously stated, due to the nature of the production, balsamic vinegar should be a rich source for the occurrence of HMF and this substance could be used as an indicator of the age. A simple HPLC method with minimum sample preparation is applied for this purpose.

## MATERIALS AND METHODS

### High-Performance Liquid Chromatography (HPLC).

The HPLC system consisted of a 1090 Hewlett-Packard device with integrated diode-array detector, injector loop, and autosampler. Data were collected using an HP XM 2 Personal Computer equipped with ChemStation version B.02.06. Data treatment was performed with a Hewlett-Packard XM2 Personal Computer. The chromatographic conditions described in the literature (Jeuring et al., 1980) were slightly modified.

**Chromatographic HPLC Conditions.** Stationary phase; hypersil ODS (250 × 4.6 mm; 5 μm); mobile phase, methanol/water (12.5/87.5; v/v), isocratic; flow rate, 1 mL/min; volume injected, 10 μL; column temperature, 40 °C; detection, UV absorption (identification: scan modus, 200–500 nm; quantification, 283 nm).

**Chemicals and Materials.** The methanol was HPLC-grade, and purified water was delivered by a Millipore water purification device. The ready-to-use eluent was degassed with helium constantly. The HMF standard was analytical-grade and obtained from Aldrich. Filters for sample preparation with a pore size of 0.45 μm and a PTFE membrane were obtained from Waters.

A stock solution of HMF was prepared with a concentration of 1230 mg/L in the mobile phase. According to the expected HMF concentration of the samples, three different calibration curves were prepared ranging between 0.12 and 24.6 mg/L, 2.12 and 106 mg/L, and 2.46 and 123 mg/L.

**Vinegar Samples.** The vinegar samples ( $n = 120$ ) were purchased in 11 Member States of the European Union (Austria, Ireland, Belgium, Portugal, Spain, France, Germany, The Netherlands, Italy, and the UK) and in Switzerland. Depending on their origin they were classified into "clear table vinegar" ( $n = 11$ ), "table vinegar with added caramel" ( $n = 12$ ), distilled malt vinegar ( $n = 3$ ), malt vinegar ( $n = 6$ ), vinegar de Jerez ( $n = 4$ ), white wine vinegar ( $n = 27$ ), red wine vinegar ( $n = 27$ ), apple vinegar ( $n = 26$ ), and vinegar essence ( $n = 4$ ). Vinegars cited in quotation marks did not always have an indication of the substrate origin. Some of the "table vinegar with added caramel" were pure spirit vinegars or mixtures of spirit and wine vinegars. In addition to the latter group, one malt vinegar sample was labeled as containing caramel (see Table 1).

The balsamic vinegar samples ("Aceto Balsamico di Modena", relative fast aging process with a maximum of 6 years,  $n$

**Table 1. HMF Content (mg/L) of Various Vinegars (Mean Relative Standard Deviations (RSD) (Mean Values for Two HPLC Measurements) Are Given at the Bottom of the Table)**

malt vinegar	sherry vinegar	white wine vinegar	red wine vinegar	apple vinegar	table vinegar with caramel	balsamic vinegar
1.6 <sup>a</sup>	13.8	0.3	0	0	0	316.4
2.0	30.3	0.3	0.3	0	0	352.3
2.0	30.4	0.3	0.3	1.5	0	446.6
2.1	34.8	0.4	0.4	1.8	0.2	447.0
3.0		0.5	0.4	1.8	0.6 <sup>b</sup>	476.5
7.3		0.5	0.5	2.4	0.8	637.4
		0.5	0.6	3.2	0.9 <sup>b</sup>	673.7
		0.7	0.6	3.4	1.8	733.3
		0.8	0.6	3.4	1.9 <sup>c</sup>	935.5
		0.8	0.8	3.7	2.3 <sup>c</sup>	942.9
		0.9	0.9	3.8	6.8 <sup>c</sup>	988.4
		0.9	0.9	4.3	9.0 <sup>c</sup>	1236.6
		1.0	0.9	4.5		1350.1
		1.0	1.0	5.8		1643.2
		1.2	1.0	6.0		1735.5
		1.3	1.0	6.1		3251.3
		1.6	1.1	7.0		
		2.1	1.3	7.0		
		2.2	1.3	7.4		
		2.2	1.4	7.5		
		2.3	1.4	7.8		
		2.3	1.6	8.1		
		3.3	1.7	12.8		
		3.8	1.7	14.3		
		5.2	2.2	15.5		
		8.7	2.4	19.2		
		21.5	10.7			
1.6%	2%	3.5%	0.6%	2.8%	1.4%	1.1%
RSD	RSD	RSD	RSD	RSD	RSD	RSD

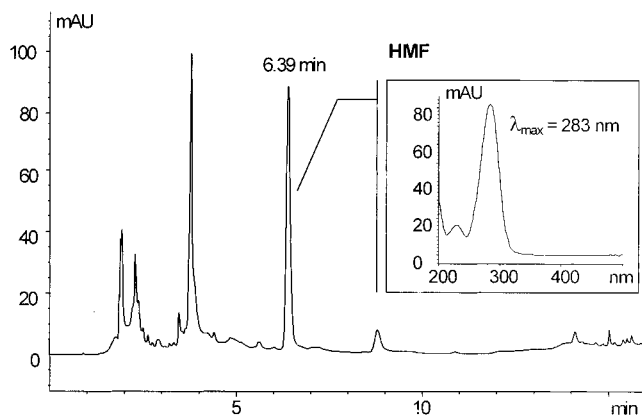
<sup>a</sup> Labeled "with addition of caramel". <sup>b</sup> Labeled as 100% spirit vinegar. <sup>c</sup> Labeled as 1/3 wine vinegar and 2/3 spirit vinegar.

= 16) were purchased in Italian supermarkets. In addition, two sets of traditionally produced balsamic vinegar ("Aceto Balsamico tradizionale") were obtained directly from the Italian producer. Each set contained 10 samples with the age increasing from 2 to 25 years. During the ripening period every 2–3 years a sample was taken from a series of barrels, and therefore the samples of each set belong to the same production batch.

**Sample Preparation.** All vinegar samples were diluted in mobile phase and filtered through membrane filters with a 0.45 μm pore size. The dilution for the vinegar samples was 1+1 (v/v) and for balsamic vinegar 1+24 (v/v). Because of the "syrup like" character of the traditionally produced balsamic vinegar these samples were weighed (100–200 mg) into a volumetric flask and dissolved in mobile phase. All of the samples were analyzed in duplicate.

## RESULTS AND DISCUSSION

The vinegar samples were analyzed in duplicate by reversed-phase HPLC using a UV diode-array detector. Though the various vinegar samples showed different separation profiles in HPLC, the resolution of HMF from other ingredients was always sufficient. In addition, the UV spectrum was recorded. A time-consuming sample preparation procedure was not necessary, and the procedure used consisted of only diluting and filtering. The relative standard deviations (RSD) ranged from 0.1 to 18%, depending on the concentrations. Quantification was performed by using an external calibration curve. Recovery rates (95–101%) were obtained by the spiking of an apple vinegar sample in the range of 2–7 mg/L.



**Figure 1.** Chromatogram of a sherry wine vinegar.

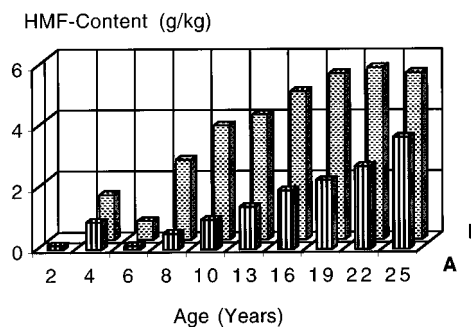
The chromatogram of a sherry vinegar with the corresponding UV spectrum of HMF is presented in Figure 1.

HMF was present in most of the samples analyzed. However, as expected, "clear table vinegar" samples, distilled malt vinegar, and vinegar essence did not contain any HMF. Such vinegar varieties are produced synthetically or by the fermentation of industrial alcohol or distilled spirits. These matrixes and therefore also the resulting vinegar samples do not contain sugars and/or proteins, and therefore the formation of HMF is unlikely.

The distribution of HMF in different vinegar samples with the exception of traditionally produced balsamic vinegar samples is presented in Table 1.

The highest concentrations were found in the sherry vinegar samples. Sherry vinegar is produced from sherry wine and subsequently aged in oak barrels. Following the so-called "solera" aging system leads to unique organoleptic properties in the final product. The production technique is a dynamic system in which fresh wine is periodically added while a part of the most-aged product is withdrawn and bottled. One possible origin of the high HMF concentrations could be cellulose degradation of the oak barrels. HMF could barely be detected in another sherry vinegar product obtained by a quick acetification process in which the vinegar had not been submitted to aging (Garcia-Parrilla et al., 1996). In comparison, considerable amounts of HMF could also be detected in sherry wines (27–1245 mg/L in sweet sherry, 0–152 mg/L in dry sherry) (Guerra Hernandez et al., 1988; Montilla Gomez et al., 1988). Another possible source of HMF is the addition of caramel which is well established in vinegar wineries (Garcia-Parrilla et al., 1996).

It has been shown that the HMF content in untreated white and red wine does not exceed a concentration of 10 mg/L (Malik et al., 1983, 1985; Laszlavik et al., 1995). The mean values were found to be in the range of 0.1–1.5 mg/L. Higher values have only been observed in dessert wine and other sweet wines (Navara et al., 1986; Montilla Gomez et al., 1988; Sigler, 1997). The HMF contents of normally produced white wines and of those which have been aged in oak barrels have been compared. It has been demonstrated that there was no significant difference (Chatonnet et al., 1992). Our results obtained for both white and red wine vinegars are in good correlation with those obtained for the wine samples from the literature. The concentrations of HMF in white and red wine vinegar samples were found



**Figure 2.** HMF content of "Aceto Balsamico di Modena tradizionale" as a function of age.

to be between not detectable and 4 mg/L. Only four samples had a concentration considerably above that range.

It has been shown that the levels of HMF are quite variable in apple juices (0–183 mg/L) (Elkins et al., 1988). That may be due to heat treatment, long storage times, or the addition of caramel. Therefore, the analysis of HMF can be a useful tool for the confirmation of suspected adulteration or overprocessing (Elkins et al., 1988). In accordance to the varying contents of HMF in apple juice, we also found amounts ranging between 1 and 20 mg/L in apple vinegar. However, in two apple vinegar samples, HMF could not be detected.

In contrast to the distilled malt vinegar in which no HMF could be determined, the HMF content in samples labeled as malt vinegar was in the range of 2–8 mg/L. It is likely that HMF was already produced in the substrate before fermentation and during the processing. Only one sample was labeled to contain caramel but showed the lowest HMF content of this group. In this case it does not seem that the caramel contributes to the total HMF content.

With the exception of three samples in which no HMF could be detected, values between 0.2 and 9 mg/L were found in table vinegars with added caramel. Two samples consisted of 100% spirit vinegar, and in this case the added caramel is the only possible source for HMF. The vinegars with the higher HMF values were all mixtures of spirit and wine vinegar. The results for wine vinegar showed that wine vinegar itself is unlikely to contribute much to the total HMF content, especially as the mixtures contain only one-third of this kind of vinegar. These differences can be interpreted as a result of the various kinds of caramel used for the vinegar production.

Balsamic vinegar is produced by a long fermentation process from the concentrated must of white grapes, with several changes of the types of wood barrels in which it is stored. The latter holds true especially in the case of the traditionally produced vinegar samples (Bortolloggi, 1993). While the values for the samples labeled as "Aceto Balsamico di Modena" were in the range of 300 mg/L to 3.3 g/L (see Table 1), those for the traditionally produced vinegar samples ("Aceto Balsamico di Modena tradizionale") clearly depended on their age and reached values of up to 5.5 g/kg after a fermentation time of 25 years. Two sets of the traditionally made balsamic vinegar were analyzed. Each set consisted of 10 samples of the same batch of vinegar with increasing ages. The results are shown in Figure 2.

These high values for HMF in balsamic vinegar could be due to several reasons: a high starting concentration

of HMF in the concentrated must, the long fermentation process, and the storage in wood barrels. HMF had been identified besides many other phenolic compounds in wood casks for wine used for vinegar aging (Galletti and Carnacini, 1995). Among other substances, the chemical composition of wood includes lignin and polysaccharides such as cellulose and hemicellulose. When an oak barrel is produced, the inner part of the staves is burnt and wetted in order to prevent breakage. This treatment leads to a partial reduction of cellulose, thus generating HMF (Villalon Mir et al., 1992). Although HMF is not present in fresh grapes, it can be formed during the juice production especially as a result of thermal stress during heat processing to which the must is subjected. Grapes seem to have a higher capacity to form HMF than other fruits. This could be due to the sugar content and composition of grape juices. It has been found that most commercial grape juices contained measurable quantities of HMF (Malik et al., 1981). An increase of the HMF content has been observed during storage time (Wucherpfenning et al., 1983). This has also been shown for apple juices as discussed above and also holds true for other fruit juices such as citrus fruit juices (Mijares et al., 1986; Lee et al., 1986, 1988; Coco et al., 1994).

The constant rise in the level of HMF due to the storage and ripening time can be clearly seen in Figure 2. It is likely that the heat treatment of the must mostly influences the formation of HMF in the final product. In addition, the concentration due to evaporation with time will also contribute to high values.

## CONCLUSION

In general, the HMF concentration in most vinegar samples has been found to be low with the exception of balsamic vinegar samples. When we compared the HMF content of the various vinegar samples from different origin, higher values for sherry and apple vinegars were found. The vinegar samples could be divided into four groups: samples with no, low, medium, and high concentration of HMF. It has been demonstrated that caramel addition is not the only source for HMF. A clear dependence of the HMF content on the age has been observed in the case of the balsamic vinegars. Therefore, the HMF concentration could be considered as a good indicator for the vinegar age. Regarding the intake of HMF, it should be stated that vinegar does not contribute significantly in comparison to other food.

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