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Heat-induced chemical modification of grape must as related to its concentration during the production of traditional balsamic vinegar: a preliminary approach

Andrea Antonelli [a,*], Fabio Chinnici [b], Francesca Masino [a]

[a] Dip. Interdisc. Sc. Agrarie, Via J.F. Kennedy 17, 42100 Reggio Emilia, Italy
[b] Dip. Sc. Alim., Via Fanin 40, 40127 Bologna, Italy

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Abstract

Occurrence and concentration of some furanic compounds during grape must concentration were studied by high-performance liquid chromatography with diode array detection. A progressive decrease of the glucose/fructose ratio was observed, along with a progressive increase of cyclic oxygenated compounds, which followed sugar concentration. Furoic acid, furfural, and 5-hydroxymethylfurfural were detected and quantified, with the latter found to be the main substance in this group. One other unknown substance with a furan-like UV spectrum was detected. Its accumulation profile was irregular, apparently scarcely influenced by sugar concentration. Two parameters that describe colour (optical absorbance at 280 and 420 nm), pH and reducing sugar content are studied.

Keywords: Aceto balsamico tradizionale; Must concentration; Cooked must; Sugar degradation; 5-Hydroxymethylfurfural

1. Introduction

For centuries, the farmers of the Emilia-Romagna region (Northern Italy) have been making traditional balsamic vinegar (TBV), the production of which has recently been briefly described elsewhere (Cocchi, Lambertini, Manzini, Marchetti, & Ulrici, 2002). TBV is a sauce obtained from cooked must by an extended ageing process (at least 12 years) in a series of barrels. During this process, a partial alcoholic fermentation and an acetic oxidation occur, while the concentration of the product increases. Once a year, product aliquots are transferred from one barrel to the next. This procedure is called *rincalzo* (i.e. topping up procedure) and the result is a dark, thick and tasty syrup. The initial step is must concentration, which is performed in open concentrators at ambient pressure and over a direct flame to give a cooked must up to 3–4 times more concentrated than the raw grape juice. This process can be quite long (up to 20 h), and the temperature is set below the must boiling point (80–90 °C), which allows a gentle simmering of the mass.

During this process, many modifications occur. Among these, many sugars are greatly modified. They can be easily metabolised by several microorganisms, as well as undergoing chemical transformation. From a theoretical standpoint, the long thermal stress, along with high acidity of the medium, should cause an evident sugar degradation. Under these conditions, the pathway to furan derivatives is well known (Belitz & Grosch, 1999). Glucose and fructose have a common 1,2-endiol intermediate, which rapidly eliminates water. Then, through two other water losses, the 1,2-endiol gives 5-hydrohymethylfurfural (HMF) as a main compound. Furthermore, fructose has another pathway of degradation, which involves the formation of a 2,3-endiol (Fig. 1) and is therefore more heat-sensitive. The general lack of aminic nitrogen compounds in grape must, together with protein coagulation as a consequence of temperature increase, makes the Maillard

* Corresponding author. Tel.: +39-0522-300531; fax: +39-0522-301482.
E-mail address: andrea.antonelli@unimo.it (A. Antonelli).

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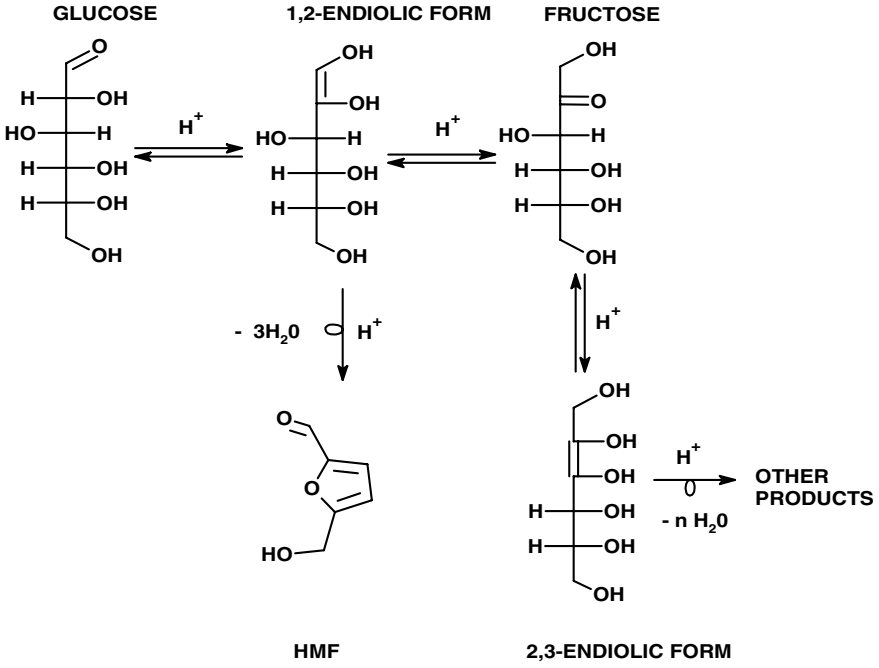

Fig. 1. Reaction pathway of 5-hydroxymethylfurfural formation from hexoses (simplified).

reaction unlikely. In addition, acidic media hinder this reaction. Caramelisation, which generally occurs at higher sugar concentration, is also unlikely to be significant. However, HMF is the main product in both Maillard reaction and caramelisation, as well as in the predominant acid degradation of sugars which under these conditions, seems to be the favourite pathway.

For these reasons, HMF is often associated with thermal stresses in foods containing sugar. It has been identified in a great variety of foods (Lee & Nagy, 1990) and has been quantified in fruit juice (Maijares, Park, Nelson, & McIver, 1986), wine (Williams, Humphreys, & Reader, 1983), honey (Salinas, Mansilla, & Nevado, 1991) and milk (Morales, Romero, & Jimenez-Pérez, 1992). In some cases, limits for HMF concentration are established by the law, for example in honey (G.U., 1982) and concentrated rectified must (Reg. CE, 1999).

A recent study on HMF in different vinegars (Theobald, Müller, & Anklam, 1998) showed that only TBV can reach levels as high as 5.5 g/kg. Lower concentrations, by up to 3 orders of magnitude, were reported for other vinegars (Fig. 2), although balsamic vinegar of Modena showed HMF contents similar to TBV (Theobald et al., 1998). Balsamic vinegar of Modena is obtained after a short ageing period from a mixture of wine vinegar and cooked must (D.U., 1965). In a previous paper on furans in TBV (Chinnici, Masino, & Antonelli, 2003), we found amounts of HMF similar to those found by others (Theobald et al., 1998), along with lower quantities of 2-furfural (FAH), 2-furoic acid (FAC), and 5-acetoxymethyl-2-furaldehyde (AMFA). These substances derive from hexose (HMF) or pentose

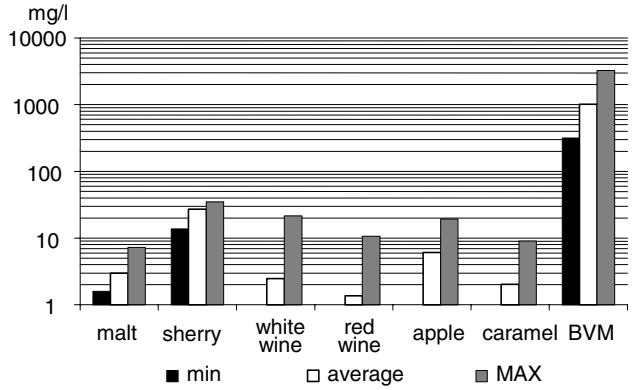

Fig. 2. Minimum, maximum, and average 5-hydroxymethylfurfural content in different vinegars. BVM: balsamic vinegar of Modena (adapted from Theobald et al., 1998).

degradation (FAH and FAC) and by acetylation of HMF (AMFA). There is no correlation between the substances themselves or with the scores given by panellists called upon to judge TBV quality and classification, although AMFA was identified and proposed as an ageing indicator by others (Giacco & Del Signore, 1997).

This even distribution of furans in TBV samples suggested that we should study the early stage of furan development: must concentration. During that stage, most of the furan content is produced, while concentration occurring during the ageing period and wood release give a further significant contribution to furan concentration.

The dietary intake of furanic compounds, and HMF in particular, is supposed to have negative effects on human health. A feeding test on rats demonstrated no adverse effect up to 450 mg/kg body weight (Lang, 1970). More recently, the acute oral LD_{50} has been reported at 2.5 g/kg in male mice (http://ntp-server.niehs.nih.gov/). This high tolerance to HMF, and the low TBV intake (few ml/meal) exclude any danger connected to regular consumption of TBV.

This paper represents an initial approach to furan development and concentration in cooked must.

2. Materials and methods

2.1. General measures

Neat samples were used for pH and °Brix measures, which were carried out with a pH meter and a refractometer, respectively.

2.2. Optical measures

Optical densities at 280 and 420 nm were measured on a Jasco (Tokyo, Japan) double beam spectrophotometer equipped with 1 cm optical path quartz cuvettes. The last two samples of each series were read with 0.1 cm path length cuvettes. Total phenols (TP) on diluted samples (1:50 w/v) were quantified as gallic acid equivalents at 280 nm, as shown after the application of an external standard curve.

2.3. Chemicals

Glucose, fructose, FAH, HMF, FAC, 2-acetylfuran and furfuryl alcohol were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Standard solutions were solubilised into the mobile phase at different concentration ranges.

A standard amount of AMFA was prepared from HMF by adding an equimolar amount of freshly distilled acetyl chloride in the presence of a pyridine excess. A diluted HCl solution was added to the crude reaction product; it was then extracted with ethyl acetate. The extract was washed with $NaHCO_3$ solution, and finally vacuum-dried. The presence and the purity (97%) of AMFA were confirmed by GC/MS analysis.

2.4. Sample preparation

All must samples were diluted in the mobile phase up to 10 times as a consequence of different solute concentrations. Before analysis, each sample was filtered through a membrane filter (0.22 μm).

2.5. High-performance liquid chromatograph

The method for furan determination in TBV was recently published (Chinnici et al., 2003) and, therefore, is only summarised here. The HPLC system consisted of Jasco (Tokyo, Japan) equipment formed by an isocratic pump, a refractive index (RI) detector for sugar quantification, a diode array detector (DAD) (PU 810), for furan detection and an injection valve Rheodyne (Cotati, CA) fitted with a 20 μl loop. Data were collected and processed by a personal computer equipped with Borwin 5.0 software (JMBS Development, Grenoble, France).

2.6. Chromatographic conditions

A Bio-Rad Aminex HPX 87H Hydrogen form cation-exchange resin-based column (300 × 7.8 mm i.d.) at 22 °C was used. The samples were separated using 0.01 N phosphoric acid and 16% acetonitrile at 0.6 ml/min as mobile phase. Furans were detected at 215 nm (furfuryl alcohol), at 254 nm (FAC) and at 280 nm, for HMF, AMFA, FAH and 2-acetylfuran.

2.7. Cooked must samples

Two different samples of Trebbiano di Romagna grape must (100 l each) were concentrated in a steel pan of 120 l capacity to about 40 l. The mass was rapidly heated up to boiling point; then the temperature was set at 80 °C for the whole process (48 h). During this time, five samples were collected at irregular intervals from each batch. The collected samples were rapidly cooled, diluted 10-fold with distilled water to prevent solute precipitation, and stored at 0 °C until analysed.

3. Results and discussion

3.1. General considerations

The concentrations of the musts gave final must volumes of 36 and 42 l for Must I and II, respectively. The two products had comparable final sugar contents (40.1 and 38.7 g/100 g), but they showed different concentration rates, as much as 2.95 times for Must I, and 2.21 for Must II (Table 1). In order to eliminate the problems arising from irregular frequency of sample withdrawals, °Brix value was used, on the assumption that this value was directly proportional to the concentration process. The identified furans, in fact, represented less than 1% of the final total sugar content.

Generally, all the analytical parameters considered increased, but at different rates. Only pH showed an opposite trend, even if it was scarcely affected by the

Table 1
Composition variation of some chemical parameters during concentration of the two musts at five sampling points

	pH	°Brix	TP[a]	OD_{420}[b]	Glucose[c]	Fructose[c]	Concentration rate[d]
Must I							
1	3.00	15.0	183	0.335	6.4	7.2	1.00
2	2.95	20.0	231	0.310	9.1	9.8	1.39
3	2.84	29.0	588	1.198	13.7	14.5	2.07
4	2.78	35.9	967	2.347	16.9	17.6	2.53
5	2.74	41.3	1144	3.048	20.0	20.1	2.95
Must II							
1	3.07	17.5	346	0.320	8.1	9.0	1.00
2	3.00	17.3	414	0.294	8.1	9.0	1.00
3	2.96	23.3	403	0.860	10.2	11.4	1.26
4	2.89	28.0	702	2.007	12.5	13.6	1.53
5	2.77	41.3	1330	3.181	18.5	19.3	2.21

[a] Total phenols; data are expressed as mg/kg of gallic acid.
[b] Optical density at 420 nm.
[c] Data are expressed as g/100 g.
[d] Based on total sugar content.

process. In fact, both musts showed a decrease of about 0.3 pH units.

Despite the sharp increase of reducing sugars, fructose and glucose showed slight but marked differences (Table 1). At the beginning of the process, the glucose/fructose ratio was about 0.89 in both cases, and the differences between the two sugars were close to 1 g/100 g. In ripe grapes this pattern is normal. However, these differences tended to disappear during the concentration process. When the musts reached 41.3 °Brix, the difference between the two sugars in Must I was as low as 0.14%, while Must II showed a less significant decrease (0.83% difference between initial and final concentration step). These considerations are particularly notable considering the relative sugar difference, i.e. fructose−glucose/fructose+glucose (Fig. 3). Especially in Must I, these trends confirm the greater instability of fructose in acid media.

The effect of must concentration on colour was studied on the basis of the OD at 280 and 420 nm. In wine, the first parameter is associated with the total phenolic content. The presence of HMF interfered with this determination, as HMF has an absorbance maximum at 283 nm. On the other hand, determination of total phenols by the Folin–Ciocalteau reagent, which is an alternative and widely accepted method, would be greatly influenced by the increasing sugar content of concentrated musts (Slinkard & Singleton, 1997). By contrast, OD_{420} represented a real measure of colour density, which gave a measure of the browning of each sample. During concentration, this last parameter increased by 1 order of magnitude in both musts, with a more irregular trend in Must II.

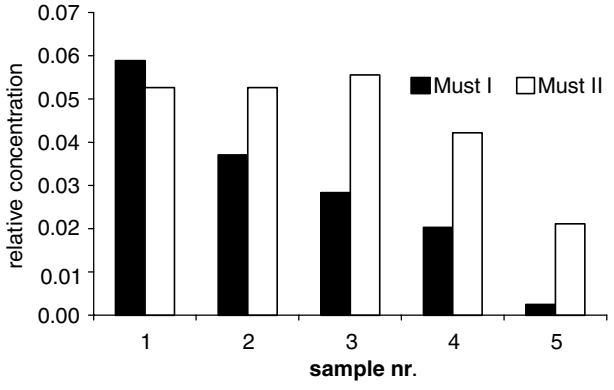

Fig. 3. Relative sugar difference (fructose−glucose/fructose+glucose) during the concentration of the two musts.

The concentration process should have ensured a low rate of oxidation. In fact, polyphenoloxidases were denatured by heat, while oxygen was stripped as a consequence of boiling. In spite of this, TP increased by factors of 6 and 4, respectively. As already explained, this sharp trend may be partially due to the accumulation of HMF, which, however, had a different accumulation profile (see Section 3.2). Moreover, the formation of phlobaphenes from must proanthocyanidins subjected to heating, should also be considered. More specific polyphenol determinations would be required to reach a more definitive answer about the fate of these substances. At the moment, only speculation is possible. In any case, good correlations between concentration (°Brix) and these two spectral data were verified (Table 1). In addition, the two straight lines that described the increase of TP were parallel, as verified by a *t*-test on the two slopes ($t_{calc} = 0.057$, $t_{stat} = 2.45$ at a 95% probability level). On the other hand, OD_{420} did not show the same trend. When more data are available, these two easy spectrophotometric determinations could represent an important tool for following the concentration process.

Except for pH, that showed quadratic correlations ($R^2 > 0.99$ and $R^2 > 0.95$ for Must I and Must II, re-

Table 2
Furan amounts (mg/kg) in the two musts during concentration

	FAC	HMF	UK1	FAH
Must I				
1	0.10	0.76	n.d.	0.01
2	0.83	78.8	70491[a]	0.32
3	1.92	1046	704587	3.68
4	2.83	2053	575186	5.61
5	3.42	3861	2032000	7.81
Must II				
1	0.10	3.32	n.d.	0.01
2	1.12	43.4	5216	0.10
3	3.40	342.7	242802	0.41
4	3.47	706	214986	1.73
5	6.83	2283	639953	4.48

FAC: 2-furoic acid; HMF: 5-hydroxymethylfurfural; UK1: peak unknown at 27.11 min; FAH: 2-furfural; n.d.: not detected.
[a] Peak area.

spectively), all other parameters showed very good linear correlations with concentration ($R^2 > 0.94$).

3.2. Furans

These compounds characterise the composition of cooked must. The products of their condensation are partially responsible for the dark colour of TBV.

The concentration process allowed the formation of FAC, HMF and FAH (Table 2), along with an unknown substance, which eluted at 27.11 min (UK1). This last compound had UV spectrum very similar to HMF (Fig. 4) and it had already been reported in our previous work on furanic compounds in TBV (Chinnici et al., 2003). Unlike other furanic compounds, UK1 did not follow a regular increase. In particular, its amount decreased between the 3rd and 4th sample. This trend was more evident in Must I (−18%) than in Must II (−11%). At the moment, no explanation is possible. To understand the origin of UK1, a 20%-fructose solution containing 5% tartaric acid was heated at 80 °C for 8 h. This period of time was long enough to generate an appreciable amount of UK1 along with HMF and FAH. For this reason, UK1 must be considered a sugar degradation intermediate, thus excluding the detection of another must constituent. Its isolation and identification are still in progress.

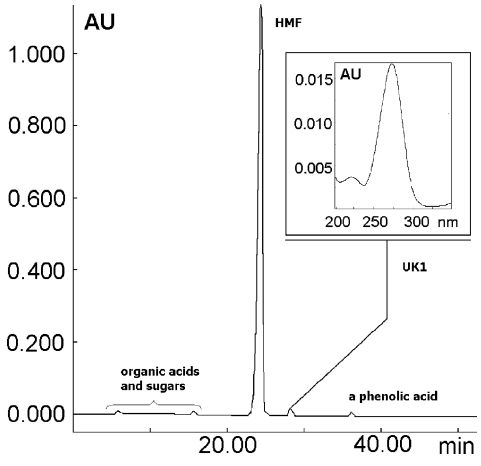

Fig. 4. HPLC trace (280 nm) of a sample (HMF: 5-hydroxymethylfurfural). The UV spectrum for peak 27.11 (UK1) is also reported.

The other peaks, which eluted before HMF, were sugars and organic acids, while the last eluting peak could be a phenolic acid, in consideration of its UV spectrum.

In our samples, AMFA was not detected, as a consequence of the lack of any microbiological activity. In fact, this substance is the acetyl derivative of HMF, which comes from acetic acid production by acetic bacteria. It is also evident that the concentration process yields most of the furan in TBV since, at the end of the concentration process, both musts had furan concentrations comparable to those of TBVs. As a consequence, wood releases seem to be negligible during TBV ageing. There are at least two reasons for this. First, barrels are generally quite old, and have been subjected to the most intense activity in the first years of their life. Second, the last casks of a set are very small (10–20 l). Their dimensions are not compatible with fire bending of the staves. For this reason, the staves of such casks were sawed, no heat was applied and no furanic compound was generated. These suppositions will be clearer in the near future, when some sets of casks for TBV production will be analysed. These results will better explain furan evolution during ageing.

At the moment, it is clear that HMF was the main product of glucose and fructose degradation, but its accumulation occurred at different rates. Generally speaking, its increase was slow up to 30 °Brix, then it continued at a higher rate (Fig. 5) just when UK1 decreased. However, the rates and the final concentrations are quite different. On a °Brix basis, the process yielded

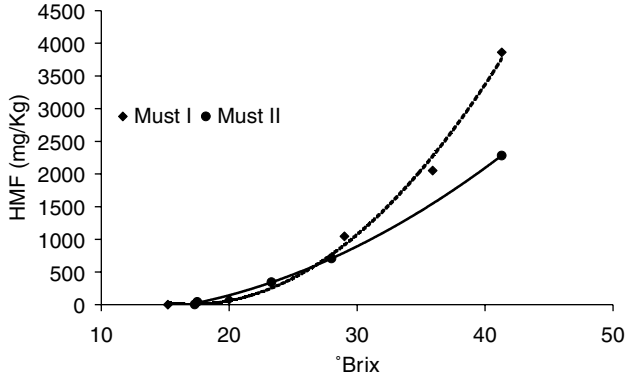

Fig. 5. Hydroxymethylfurfural (HMF) concentrations (figures) and regression curves during the concentration of the two musts ($R^2 > 0.99$ in both cases).

a concentration of 2.75 and 2.36 times for Must I and Must II, respectively. It is surprising that such a slight difference in concentration gave an HMF difference of 1578 mg/kg. On the other hand, HMF seemed to increase just at the end of the process (Fig. 5), when the sugar concentration was higher. As a consequence, quadratic correlations were found ($R^2 > 0.99$ in both cases).

HMF determination, performed upon a single sample kindly given by a local supplier of concentrated must, showed the importance of the thermal process. In fact, this sample was concentrated at reduced pressure and with double effect equipment. Under these conditions, its HMF content was as low as 7 mg/kg even if the final concentration of this must reached 61 °Brix. However, it is important to stress that such a concentration technique is not in accordance with the traditional procedure of TBV production (G.U., 2000).

The other furans showed linear accumulation trends (Table 2), with very high determination coefficients ($R^2 > 0.94$). FAC and FAH profile showed opposite trends. They were present in very low concentrations at the beginning of the process, then they increased up to 3.42 and 7.81 mg/kg in Must I, respectively. In Must II, by contrast, FAC reached 6.83 mg/kg, while FAH was quantified at only 4.48 mg/kg. These different behaviours are mainly attributable to different pentose contents in the raw material.

4. Conclusions

Although this study represents only an initial approach, it is evident that furan compounds in general, and HMF in particular, are excellent parameters in the study of cooked must quality. Compared to many other thermally processed foods, in this kind of must high furan quantities are not necessarily bad. In fact, TBV is a typical product, the characteristics of which are mostly due to cooked must.

On the other hand, HMF accumulation kinetics in cooked must as well as the influence of grape maturation (i.e. sugar and acid quantities), require more studies. In addition, time, temperature and volume can be easily controlled only with laboratory concentration equipment. Further studies will examine these aspects.

References

Belitz, H.-D., & Grosch, W. (1999). *Food chemistry* (second ed., pp. 252–261). Berlin: Springer-Verlag.

Chinnici, F., Masino, F., & Antonelli, A. (2003). Determination of furanic compounds in traditional balsamic vinegars by ion-exclusion liquid chromatography and diode array detection. *Journal of Chromatographic Science, 26*, 2161–2171.

Cocchi, M., Lambertini, P., Manzini, D., Marchetti, A., & Ulrici, A. (2002). Determination of carboxylic acids in vinegars and in aceto balsamico tradizionale di modena by HPLC and GC methods. *Journal of Agricultural and Food Chemistry, 50*, 5255–5261.

D.U. 3 December 1965.

G.U. no. 288, 19 October 1982, law no. 753.

G.U. no. 124, 30 May 2000.

Giacco, M., & Del Signore, A. (1997). Il contenuto di 5-acetossimetil-2-furaldeide nell'aceto balsamico tradizione di Modena e di Reggio Emilia. *Rivista di Merceologia, 36*, 91–97.

http://ntp-server.niehs.nih.gov/.

Lang, K. (1970). Influence of cooking on foodstuffs. In G. H. Bourne (Ed.), *World review of nutrition and dietetics* (Vol. 12, pp. 266–317). Basel: S. Karger AG.

Lee, H. S., & Nagy, S. (1990). Relative reactivity of sugars in the formation of 5-hydroxymethylfurfural in sugar-catalyst model system. *Journal of Food Processing and Preservation, 14*, 171–178.

Maijares, R. M., Park, G. L., Nelson, D. B., & McIver, R. C. (1986). HPLC analysis of HMF in orange juice. *Journal of Food Science, 51*, 843–844.

Morales, F. J., Romero, C., & Jimenez-Pérez, S. (1992). An enhanced liquid chromatography method for 5-hydroxymethylfurfural determination in UHT milk. *Chromatographia, 33*, 45–48.

Reg. CE no. 1493/99, 17 May 1999.

Salinas, F., Mansilla, A. E., & Nevado, J. J. B. (1991). Flow-injection determination of HMF in honey by the Winkler method. *Fresenius Journal of Analytical Chemistry, 340*, 250–252.

Slinkard, K., & Singleton, V. L. (1997). Total phenol analysis: automation and comparison, with manual methods. *American Journal of Enology and Viticulture, 28*, 49–55.

Theobald, A., Müller, A., & Anklam, E. (1998). Determination of 5-hydroxymethylfurfural in vinegar samples by HPLC. *Journal of Agricultural and Food Chemistry, 46*, 1850–1854.

Williams, M. A., Humphreys, R. C., & Reader, H. P. (1983). The analysis of 5-hydroxymethylfurfural in Port by high performance liquid chromatography. *American Journal of Enology and Viticulture, 34*, 57–60.