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Evolution of 5-hydroxymethylfurfural (HMF) and furfural (F) in fortified wines submitted to overheating conditions

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ABSTRACT

As furfural (F) and 5-hydroxymethylfurfural (HMF) are essentially formed from sugar dehydration, especially in food submitted to heat, they can be found in beverages, as well as fortified sweet wines. In order to assess the impact of temperature on Madeira winemaking, three traditional varieties of Madeira wines (*Malvasia*, *Sercial* and *Tinta Negra Mole*) were studied to evaluate the F and HMF contents. The wines were produced by two vinification processes, following traditional and modern methodologies, heated at standard conditions (30 °C and 45 °C, for 4 months) and compared with the same wines submitted to overheating conditions (55 °C, for 4 months). The RP-HPLC-DAD methodology used for the control of F and HMF during the process showed no significant changes in the wines maintained at 30 °C (*canteiro*) and a noticeable but controlled increase in the wines heated at 45 °C (*estufagem*) where values up to about 150 mg/L of HMF could be found in sweet wines. The strong relation of this compound with the sugar content and baking temperature stood out in the wines submitted to overheating conditions where values higher than 1 g/L could be found for sweeter wines, with HMF level being in general higher than F.

The results clearly suggest that the amount of HMF in these fortified wines can be easily controlled when submitted to adequate conditions of heating during *estufagem* and storage. Furthermore, different temperatures for the baking of sweet and dry wines may be considered.

1. Introduction

Madeira wines are classified as fortified, with alcoholic strengths between 17 and 22% (v/v) and sweetness levels ranging from 0 (dry) up to about 130 g/L (sweet). Fermentation is carried out according to both the grape variety involved and the type of wine being produced (dry, medium dry, medium sweet and sweet). *Malvasia* and *Sercial* grapes are two of the traditional white varieties used for the preparation of high quality sweet and dry wines, respectively and *Tinta Negra Mole* is a red grape versatile variety, being used for the production of different types of Madeira. Sweet wines, traditionally not fermented, are currently obtained by a partial fermentation, in order to ensure 4% of alcohol exclusively derived from alcoholic fermentation and maintaining the high content of residual sugars. In contrast, Madeira dry wines can be completely fermented to sugar levels close to 0 g/L (traditional method) or be fermented to low sugar levels (less than 1.5 °Be). Modern vinification techniques, following recent studies carried out to improve the typicity characteristics (Oliveira e Silva et al., 2008), have been introduced with the purpose of stabilizing the total sugar content in sweet wines to about 80 g/L and maintaining some residual sugars in dry wines. When the required sweetness level is attained the fermentation is stopped by the addition of a natural grape spirit (containing 95% (v/v) of ethanol). Then, two ageing processes can be followed: the *canteiro*, usually applied to the finest wines, namely those produced from *Malvasia* and *Sercial* grapes, where the wines are maintained under mild heating storage conditions (heating rooms not exceeding 30 °C); and the *estufagem*, where the wines are heated to about 45 °C up to 3 months. The *Tinta Negra Mole* red variety, the most prolific variety in Madeira, used for the production of wines with different sweetness, is usually submitted to the practice of *estufagem* before undergoing a normal maturation process in oak casks for a minimum period of 3 years. During the heating stage, a premature ageing process occurs, originating the typical colour and bouquet of these wines and contributing to their exceptional longevity.

The current concern with the alimentary quality increases the necessity for the use of chemical markers, which evaluate possible damages in the foodstuffs submitted to overheating and drawn out storage. The heating process can be used advantageously to preserve foods, destroying the spoilage organisms, but holding back the

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nutritional and organoleptic properties. In the case of Madeira wines, the heating process, used in the preparation of these wines since the 18th century, is generally associated with the toasted aroma and typical brownish colour. Among the aromas formed during this period (Oliveira e Silva et al., 2008), the current study has focused its attention on the formation of two furanic compounds, furfural (F) and 5-hydroxymethylfurfural (HMF). These are the main degradation products of carbohydrates and their occurrence in foods is generally related to non-enzymatic browning reactions, namely Maillard type reactions (MR), sugar degradation in acid medium and caramelization (Antonelli, Chinnici, & Masino, 2004; Granados, Mir, Serrana, & Martinez, 1996). Indeed, they are currently used as heat-treatment markers of foods.

In acidic medium, the heating of pentoses and hexoses originates F and HMF, respectively, after a slow enolization and a fast β-elimination of three water molecules (Belitz, Grosch, & Schieberle, 2009). Indeed, the acid-catalysed degradation mechanism of fructose and glucose produces in a first step 1,2- or/and 2,3-enediolic intermediates, which rapidly eliminates water molecules before producing HMF (see Fig. 1, adapted from Antonelli et al. (2004)). At wine pH (about 3.5) the formation route for F and HMF in Madeira wines can be explained almost entirely by acid-catalysed sugar degradation, since Maillard chemistry is not favoured in the acidic media. The analytical control of F and HMF has received some importance and its occurrence has been reported in several food products, including fruit juices (Gökmen & Acar, 1999), beers (Lo Coco, Valentini, Novelli, & Ceccon, 1995), brandies (Granados et al., 1996) and fortified wines (Cutzach, Chatonnet, & Dubourdieu, 1999; Ho, Hogg, & Silva, 1999). From a safety perspective and for food quality assurance, HMF legal limits were already issued for some foodstuffs, namely for concentrated rectified grape must: EC Regulation No. 1493/99 sets up a limit of 25 ppm (Falcone, Tagliazucchi, Verzelloni, & Giudici, 2010). The F content is also useful as an off-flavour indicator and HMF is frequently correlated with browning reactions (Lo Coco et al., 1995).

Being essentially considered as indicators of overheated foodstuff, the presence of HMF and F in foods has raised some toxicological concerns in recent years. Some authors considered that they are natural components of traditional foods, posing no risk to human health (Adams et al., 1997; Janzowski, Glaab, Samimi, Schlatter, & Eisenbrand, 2000), while others say that HMF can be poisonous to the nervous system due to accumulation in the body when combined with proteins, eventually causing damages in the muscles and viscera (Li & Lu, 2005). HMF derivatives, such as 5-chloromethyl- and 5-sulfoxymethylfurfural (SMF), have been associated with cytotoxic, genotoxic, and tumoral effects (Nassberger, 1990; Surh, Liem, Miller, & Tannenbaum, 1994; Zhang et al., 1993). In recent studies, special attention has been given to HMF-related carcinogenicity (Monien, Frank, Seidel, & Glatt, 2009; Durling, Busk, & Hellman, 2009).

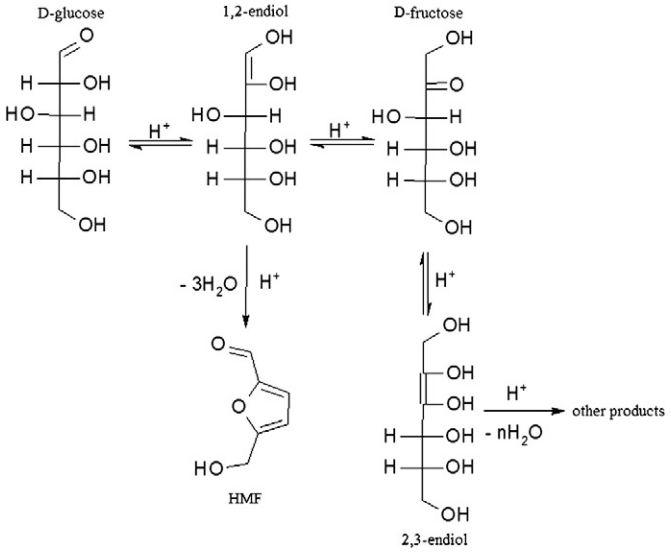

Fig. 1. HMF formation pathway by sugar acid-catalysed dehydration (adapted from Antonelli et al. (2004)).

The growing attention of the scientific community towards the potentially toxic effects of HMF and F has triggered the current interest on the formation of these compounds in Madeira wines, especially because sweet wines have a rather high content of carbohydrates and are submitted to a quite long heating process (at least 3 months).

The study was focused on their determination in wines with different sweetness levels, produced under diverse fermentation and heating conditions, in order to simulate different ageing processes. To do so, three traditional varieties of Madeira wines, *Malvasia*, *Sercial* and *Tinta Negra Mole*, were produced by two different vinification processes and heated under overheating conditions (at 55 °C for 4 months), and compared with wines submitted to standard heating conditions (30 and 45 °C). F and HMF levels were determined by direct RP-HPLC-DAD analysis of the wines under study.

2. Experimental

2.1. Reagents

HMF and F analytical standard-grade (both with assay >98%) were obtained from Acros Organics (Geel, Belgium). D-fructose and D-(+)-glucose were supplied by Himedia (Mumbai, India) with assays higher than 99%. The hydroalcoholic solutions were prepared with ethanol (96%) from Sigma-Aldrich (St. Louis, MO, USA) and ultra-pure water (Milli-Q System, Millipore, Bedford, MA, USA). The chromatographic mobile phases were prepared with ultra-pure water, methanol HPLC grade (Sigma-Aldrich, St. Louis, MO, USA) and acetic acid (JMGS, Portugal, >99%). All solvents used were previously filtered through 0.45 μm membranes from Pall Corporation (Ann Arbor, MI, USA) to remove any impurities.

2.2. Wines

Traditionally, the vinification process of sweet Madeira wines used to be characterized by short fermentative steps or even by its absence, originating wines with high sugar levels, whereas dry Madeira wines used to be completely fermented (traditional methods). Nowadays, there is a tendency to extend the fermentation of sweet wines (lowering the amount of residual sugars) and shorten the fermentation of dry wines (modern methods).

For the purpose of the present study, about 600 L of must was obtained from *Malvasia* grapes (2003 harvest) and equal amounts were fermented according to different methods: traditional and modern. One was almost not fermented, the *Malvasia* traditional wine (*Mt*), containing 125 g/L of residual sugars. The other one was slightly fermented (4 days at 21 °C), getting a sugar level of about 78 g/L, denominated as *Malvasia* modern wine (*M*m). The same procedure was applied to produce sweet wines from *Tinta Negra Mole* grapes: *Tinta Negra Mole* modern sweet (*T*mS) and *Tinta Negra Mole* traditional sweet (*T*tS). Two *Sercial* wines (equal amounts) were produced from 600 L of must. One was fermented until complete transformation of sugars (*Sercial* traditional, *St*). The other was fermented maintaining a low level of residual sugars (*Sercial* modern, *Sm*). Similarly, *Tinta Negra Mole* was used for the production of two dry wines: *Tinta Negra Mole* modern dry (*T*mD) and *Tinta Negra Mole* traditional dry (*T*tD). All wines were industrially elaborated in stainless steel tanks of local Madeira wine-producing cellars and the alcoholic fermentation was carried out by indigenous yeast under controlled temperature and malolactic fermentation was not encouraged. Sulphite was added to musts up to 150 mg/L. After vinification, all wines were placed in stainless steel vats and heated at three