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Assessment of the development of browning, antioxidant activity and volatile organic compounds in thermally processed sugar model wines

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ABSTRACT

The study evaluates the contribution of the fructose and glucose's degradation for the Madeira wine's features. The browning index, antioxidant activity and volatile organic compounds developed by the glucose and fructose model systems simulating thermally processed sweet Madeira wines were assessed. Sixteen different fructose/glucose model systems were prepared in synthetic wine and stored at 50 °C for 4 months. Then, three model wines were also submitted to 70 °C for 1 month. The browning index and the antioxidant activity ranged between 0.00 and 0.27 AU and 3.0–65.3 mg(GAE)/L, respectively. The development of several volatile organic compounds was demonstrated (up to 47). The identified compounds were mostly furans, with 5-hydroxymethylfurfural as the most abundant. For the first time, it was shown that the origin of sotolon in sweet wine can be associated with the acid-catalyzed fructose degradation mechanism. Other 2(5H)-furanones were also identified. It could be concluded that part of the browning, antioxidant activity and aroma compounds developed in sweet fortified wines is associated with the thermal degradation of fructose in acid medium.

1. Introduction

It is generally accepted that sugar in acidic media can be degraded into several low-molecular weight compounds, such as furans and pyrans (Belitz, Grosch, & Schieberle, 2009, pp. 248–339). Additionally, brown-colored compounds can also be formed. Parallel to the sugar degradation reaction in acidic medium, Maillard reaction can also occur, leading to similar products. This complex reaction is also known to develop browning due to a series of subsequent and parallel reactions between carbonyl compounds (such as sugars) and free amino groups (usually amino acids). Maillard reaction is very important in food quality, mainly in heat-processed foods, affecting not only color but also the flavor and nutritional value. It can produce high antioxidant activity products, namely melanoidins, although in contrast, can eventually have toxicological implications, such as acrylamide formation (Osada & Shibamoto, 2006; Yilmaz & Toledo, 2005). In general, the sugar type determines the flavor compounds formed and the amino acids affect the kinetics (van Boekel, 2006).

Considering that this kind of reactions develop complex intermediates and final reaction products, researchers commonly use model systems to perform their studies. These studies usually use water as solvent and only a limited number of reports have dealt with hydro alcoholic systems (Pripis-Nicolau, de Revel, Bertrand, & Maujean, 2000; Shen & Wu, 2004; Shen, Tseng, & Wu, 2007). Shen and Wu (2004) used ethanolic systems and proved that the browning extent and the 5-hydroxymethylfurfural (HMF) content rise with the ethanol increase. They also found different product profiles in aqueous and ethanolic model systems indicating some differences in the reaction mechanisms (Shen et al., 2007). Furthermore, there are some studies that highlight the formation of flavor components in model wine systems, at low pH and temperatures. Sanz and Martínez-Castro (2009) and Kroh (1994)

Abbreviations: ABTS, 2,2′-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid; Arg, L-arginine; Asp, L-aspartic acid; Cys, L-cysteine; Fru, D(−)-fructose; Glc, D(+)-Glucose; GAE, gallic acid equivalents; GABA, γ-aminobutyric acid; HMF, 5-hydroxymethylfurfural; TAA, total antioxidant activity; VOCs, volatile organic compounds.

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studied model wine systems containing glucose with alanine, arginine and proline. Pripis-Nicolau et al. (2000) reported the reaction of carbonyls (acetoin and acetol) and dicarbonyls (glyoxal, methylglyoxal, diacetyl and pentan-2,3-dione) with 14 amino acids. They found that this reaction leads to the formation of many products, including pyrazines, methylthiazoles, acetylthiazoles, acetylthiazolines, acetylthiazolidines, trimethyloxazole, and dimethylethyloxazoles, especially due to the presence of cysteine, through decarboxylation and participation in the Strecker degradation mechanism. Moreover, they also found that these compounds have a remarkable odor, with notes resembling sulfur, corn, pungent, nut, popcorn, roasted hazelnut, toasted, roasted, and ripe fruits. Later, Marchand, de Revel, Vercauteren, and Bertrand (2002) have proved the occurrence of a Maillard intermediate (*N*-(2-sulfanylethyl)-2-oxopropanamide) in the formation of 2-acetylthiazole from methylglyoxal and cysteine using a model wine system. Recently, Pons, Lavigne, Landais, Darriet, and Dubourdieu (2010), used model wine solutions at 40 °C during 6 months for testing the ability of several reported precursors to produce sotolon under very different experimental conditions.

Considering that Maillard reaction takes place as low as 50 °C, favored at pH 4–7 and that caramelization, even if it requires higher temperatures, is favored at pH 3–9 (Kroh, 1994; Morales & Jiménez-Pérez, 2001), it is reasonable to admit that both reactions can eventually occur during the heating process traditionally applied to the Madeira wines (up to 50 °C during at least 3 months) – *estufagem* – and contribute to their browning and flavor. Madeira wine processing is described elsewhere (A.C. Pereira et al., 2016; V. Pereira, Albuquerque, Ferreira, Cacho, & Marques, 2011). These wines hold an alcohol by volume between 17 and 22% (v/v: mL ethanol/100 mL wine) and are produced in different styles, namely dry (total sugars: 49.1–64.8 g/L), medium-dry (64.8–80.4 g/L), medium-sweet (80.4–96.1 g/L), and sweet wines (>96.1 g/L) (IVBAM). Previous reports indicate that the total amino acid content, which can vary between 644 and 178 mg/L, can decrease up to 30% after being submitted to *estufagem* (V. Pereira, Pereira, Pérez Trujillo, Cacho, & Marques, 2015).

The aim of the current study was to evaluate the contribution of fructose and glucose thermal degradation for the Madeira wine features, assessing the browning index, antioxidant activity and volatile organic compounds (VOCs) of 16 different glucose and fructose model systems, prepared under the same conditions of baked sweet Madeira wines. The role of four amino acids, namely arginine (Arg), cysteine (Cys), γ-aminobutyric acid (GABA) and aspartic acid (Asp), was also studied, taking into consideration either their abundance in Madeira wine or their relevance in terms of reactivity (V. Pereira et al., 2015; V. Pereira, Pontes, Câmara, & Marques, 2008). Additionally, 3 model systems, prepared with fructose (Fru), fructose + cysteine (FruCys) and glucose (Glc), under overheating conditions (70 °C for 1 month) were also studied in order to accelerate the development of volatiles, which might be formed from eventual hot points of the heating coil fitted in the stainless steel tank, in which the wine thermal processing is performed. As far as we know, this is the first study that seeks to find the contribution of sweet fortified wine main sugars for the browning, antioxidant activity and aromas development.

2. Material and methods

2.1. Chemicals

Glc, Fru and *L-(+)*-tartaric acid were obtained from Merck Co. (Darmstadt, Germany), the amino acids Arg, Cys, Asp were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and γ-aminobutyric acid (GABA) was supplied by Fluka BioChemika AG (Buchs, Switzerland). Ethanol was obtained from Panreac (Barcelona, Spain). Ethyl acetate was supplied by Lab-Scan (Dublin, Ireland). All chemicals had a purity grade higher than 98%.

2,2′-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) in the crystallized diammonium salt form (≈98.0%), gallic acid monohydrate (≥98.0%) and potassium persulfate were obtained from Fluka BioChemika AG (Buchs, Switzerland).

2.2. Preparation of model systems

For the preparation of the model systems, four amino acids were chosen: Arg, Cys, GABA and Asp, since they are important amino acids present in Madeira wines, shown in previous studies (V. Pereira et al., 2015; V. Pereira et al., 2008). The selected sugars were Fru and Glc, as they are the main sugars present in sweet Madeira wines. The model systems were prepared as described in Table 1. All model systems were prepared in synthetic wine (sweet Madeira wine typical conditions) containing 6 g/L of tartaric acid, 180 mL/L of ethanol and pH adjusted to 3.5, with a 1 mol/L NaOH solution. The first nine model systems intended to induce the Maillard reaction, the following three to induce the acidic degradation of sugar and the last four intended to ascertain the influence of the amino acid concentration. About 100 mL of each model system were placed into 100 mL Schott Duran® wide mouth bottles, remaining a small head space volume similar to the *estufagem* process procedure. The model systems were stored at 50 ± 0.5 °C in a Memmert UFE 400 oven (Schwabach, Germany) for 4 months to simulate the *estufagem* process (regulated conditions).

Other Fru, FruCys and Glc model systems were then submitted to overheating conditions (70 °C during 1 month) in order to identify the VOCs that clearly result or enhance from the rise of the heating temperature. Duplicates of all model systems were prepared.

2.3. Browning index

The browning index was evaluated by spectrophotometry, reading the absorbance at 420 nm ($A_{420\ nm}$) of each model systems against distilled water, after 4 months of heating. The readings were recorded on a Perkin Elmer Lambda 2 (Waltham, MA, USA) spectrophotometer using a 1 cm path length quartz cell. All samples were analyzed in triplicate.

2.4. Total antioxidant activity

Total antioxidant activity (TAA) determination, was based on the method reported by Re et al. (1999), according to the reaction of each model system with a stable ABTS radical cation ($ABTS^{\cdot+}$). Briefly, $ABTS^{\cdot+}$ was obtained by the reaction of 2 mmol/L ABTS diammonium salt with 70 mmol/L potassium persulfate in 50 mL of phosphate buffered saline (PBS). The mixture was left to stand in the dark at room temperature for about 16 h before use. For the antioxidant activity evaluation, the $ABTS^{\cdot+}$ solution was diluted with PBS to obtain the absorbance of 0.800 ± 0.030 at 734 nm. Then 12 μL of each model system were mixed with 3 mL of $ABTS^{\cdot+}$ solution. The absorbance was recorded at room temperature during 20 min. PBS solution was used as blank. The percentage of decrease of the absorbance at 734 nm was calculated by the formula $I = [(A_{blank} - A_{model\ system})/A_{blank}] \times 100$, where I = $ABTS^{\cdot+}$ inhibition (%), A_{blank} = absorbance of the blank sample (t = 0 min), $A_{model\ system}$ = absorbance of the tested model system at the end of the reaction (t = 20 min). The results were expressed as mg/L of gallic acid equivalents (GAE), by means of the following calibration curve: $I = 1.243\ GAE^{0.801}$ ($R^2 = 0.994$, between 1 and 150 mg/L).

Table 1
Conditions, browning index ($A_{420\ nm}$) and total antioxidant activity (TAA), expressed as mg/L of gallic acid equivalents (GAE), of the prepared model wines. The results are expressed as mean value (n = 6) ± standard deviation.

Model System	Sugar (g/L)	Amino acid (mg/L)	Heating conditions	$A_{420\ nm}$ (AU)	TAA (mg(GAE)/L)
GlcArg	Glucose, 125	Arginine, 100	50 °C during 4 months	0.01 ± 0.00	8.8 ± 0.3
GlcCys		Cysteine, 100		0.03 ± 0.00	13.2 ± 0.4
GlcGABA		GABA, 100		0.01 ± 0.00	3.0 ± 0.2
GlcAsp		Aspartic acid, 100		0.01 ± 0.00	3.8 ± 0.3
FruArg	Fructose, 125	Arginine, 100		0.19 ± 0.01	65.3 ± 9.5
FruCys		Cysteine, 100		0.21 ± 0.01	40.4 ± 0.7
FruGABA		GABA, 100		0.18 ± 0.01	61.0 ± 0.0
FruAsp		Aspartic acid, 100		0.12 ± 0.00	58.8 ± 3.6
FruGlc(4aa)	Fructose, 62.5 Glucose, 62.5	Arginine, 25 Cysteine, 25 GABA, 25 Aspartic acid, 25		0.09 ± 0.01	28.8 ± 0.3
Glc	Glucose, 125	–		0.00 ± 0.00	3.3 ± 0.3
Fru	Fructose, 125	–		0.14 ± 0.01	61.7 ± 0.5
FruGlc	Fructose, 62.5 Glucose, 62.5	–		0.06 ± 0.00	33.0 ± 1.0
FruArg(×2)	Fructose, 125	Arginine, 200		0.19 ± 0.01	62.1 ± 2.5
FruCys(×2)		Cysteine, 200		0.27 ± 0.01	44.9 ± 0.1
FruGABA(×2)		GABA, 200		0.19 ± 0.01	59.4 ± 0.1
FruAsp(×2)		Aspartic acid, 200		0.11 ± 0.01	54.9 ± 6.3
Fru	Fructose, 125	–	70 °C during 1 month	–	–
FruCys	Fructose, 125	Cysteine, 100		–	–
Glc	Glucose, 125	–		–	–

2.5. Volatile organic compounds

The liquid-liquid microextraction procedure was adapted from Ortega, López, Cacho, and Ferreira (2001). 5 mL of each model system were added to 3 mL of distilled water. Then, 2 g of ammonium sulfate and 5 μL of the internal standard (500 mg/L of 3-octanol, prepared in synthetic wine) were added. The extraction was carried out with 1 mL of ethyl acetate. This mixture was mechanically agitated for 30 min and finally the extract was separated from the aqueous phase and analyzed.

The gas chromatography mass spectrometry (GC-MS) analysis was carried out on a TRACE GC Ultra gas chromatograph equipped with the ISQ single quadrupole (electron impact mode) and the TriPlus autosampler (liquid mode) from Thermo Scientific (Hudson, NH, USA). The column was a DB-WAXetr 30 m × 0.32 mm i.d., with 0.5 μm film thickness from Agilent J&W (Folsom, CA, USA). The carrier gas was helium (helium N60, Air Liquid, Algés, Portugal) at 1 mL/min and 1 μL of extract was vaporized into the injector port at 250 °C, in splitless mode (1 min). The oven temperature was then raised from 40 to 220 °C at 3 °C/min and finally held at 220 °C for 5 min. The quadrupole ion source and the transfer line temperatures were maintained at 230 and 250 °C, respectively. The ionization energy was set to 70 eV. The mass range 30–300 m/z was recorded in full-scan mode.

The identification of the compounds was made by comparing their mass spectra with those of authentic compounds, when available, and with those present in the NIST08 and Wiley 6.0 mass spectra library databases. Additionally, the Kovats indexes (KI) were determined using an alkane solution (C7–C30), obtained from Supelco (Sigma Aldrich, St. Louis, MO, USA), analyzed under the same chromatographic conditions. The obtained Kovats indexes were compared with those stated on NIST Chemistry Web Book (Stein, 2008). The replicates of the model systems were analyzed in triplicate. The total ion current (TIC) was used for semi-quantitative purposes.

2.6. Data processing

All model systems were prepared in duplicate and the analysis was performed in triplicate. Significant differences were evaluated by the analysis of variance (one-way ANOVA with Holm-Sidak method) using the statistical software SigmaPlot 12.0.

3. Results and discussion

3.1. Browning index

At the initial stage, all model systems were colorless. However, throughout the heating step they became yellowish. The browning index values of the first 16 model systems range between 0.00 and 0.27 AU, which can represent up to 20–30% of the browning developed by sweet Madeira wines submitted to *estufagem* (Carvalho, Pereira, Pereira, Pinto, & Marques, 2015; V.; Pereira, Albuquerque, Cacho, & Marques, 2013). The Fru model systems (0.06–0.27 AU) presented higher absorbance after the thermal processing, when comparing with the Glc model systems (0.00–0.14 AU) – Table 1. Indeed, the browning index was in average 6-fold higher. Furthermore, the presence of amino acids tends to favor browning (Table 1), perhaps by the simultaneous occurrence of Maillard reactions and acidic sugar degradation. These results suggest that the added amino acids participate in the formation of brown Maillard reaction products. When the amino acid content was doubled the $A_{420\ nm}$ was only more pronounced in model systems containing Cys. Indeed, FruCys and FruCys(×2), attained higher browning indexes (Table 1) when comparing with those model systems prepared with the remaining amino acids.

3.2. Total antioxidant activity

The antioxidant activity of Maillard products has been studied by several scientific researchers (Chawla, Chander, & Sharma, 2007; Moreno, Peinado, & Peinado, 2007; Osada & Shibamoto, 2006; Yilmaz & Toledo, 2005). All model systems showed antioxidant activity (Table 1). TAA varied from 3.0 mg/L (GlcGABA) to 65.3 mg/L (FruArg). The Fru model systems presented 9-fold higher antioxidant activity (in average 56.4 mg/L) than the Glc model systems (about 6.4 mg/L), indicating that antioxidant activity depends on the type of sugar. Additionally, minor differences were found

between the model systems with or without amino acids, or even between the different amino acids, suggesting that antioxidant activity is mostly derived from products formed from acidic degradation of sugars.

V. Pereira et al. (2013) reported that Madeira wines after being submitted to the heating step present an antioxidant activity ranging from about 154 to 198 mg(GAE)/L, using the same assay. Thus, sugar degradation products are likely to represent about a third of the Madeira wine antioxidant activity.

3.3. Volatile organic compounds

After analysis of the ethyl acetate extracts by GC-MS, the chromatographic peaks were integrated and the relative areas (using the internal standard peak area) were calculated. Fig. 1 shows a typical chromatogram.

Twenty five VOCs were identified in the model systems heated at 50 °C during 4 months (Table 2) and forty four in those that were submitted to overheating (70 °C during 1 month, Table 3), most of them furans and ethyl esters.

It could be inferred that sugars determine the formation of VOCs rather than amino acids. In acid medium, Fru opens its ring structure easier than Glc (Robyt, 1998; Semchyshyn, 2013) and therefore is more reactive. This can explain the fact that the Fru model systems have developed a larger number of VOCs with higher abundance (up to 41) compared with those formed in Glc model systems (up to 20 - Tables 2 and 3). Gögüs, Bozkurt, and Eren (1998) also found that Fru was more reactive than Glc when studied the kinetics of Maillard reactions through the preparation of model systems mimicking boiled grape juice, with Fru, Glc, glutamine and Arg. Thus, the development of a higher number of VOCs, but also the higher browning intensity, indicates that Fru, when remains in wines, is chemically more reactive than Glc.

Nursten (1981) verified that sugars by themselves show similar reactions to those produced between sugars and amino acids. Indeed, the current study showed that model systems with or without amino acids developed similar compounds, regardless of the type of amino acid (Tables 2 and 3). Moreover, the change in the amino acid content did not reveal a significant effect in the VOCs formed (Table 2). Consequently, from the VOC analysis it was difficult to elucidate if the Maillard reaction takes place. At this pH, the Maillard pathway more likely to happen is the 1,2-enolization, which gives essentially the same products of acidic sugar degradation. On the other hand, the increase of the heating temperature revealed an increase in the number of produced VOCs, especially in Glc model system (from 5 to 20).

Most of the identified VOCs (precisely 32/47) were already identified in Madeira wines submitted to *estufagem* (V. Pereira, Cacho, & Marques, 2014), suggesting that their occurrence in Madeira wine might be derived from the degradation of sugars, mostly from Fru. In addition, other compounds present in wines, especially carbonyl compounds derived from fermentation, may interact with sugar derived-products and form other compounds usually associated with the Maillard reaction.

Furans represent about 89% of the total compounds formed in the model systems containing Fru, being HMF the most representative (about 88%). Fru is an important factor in the formation of HMF, given that, for example, FruGlc(4aa) and FruGlc, with the same amount of Fru (62.5 g/L) and Glc (62.5 g/L) showed higher amounts comparatively to the Glc model system, prepared with 125 g/L of Glc (Table 2). Table 3 shows similar results, reinforcing the previous statement. These results also support the idea that Fru in wine is more reactive than glucose. The predominance of furans can be explained by the fact that the heating of hexoses in an acid medium leads, after enolization, to the elimination of water

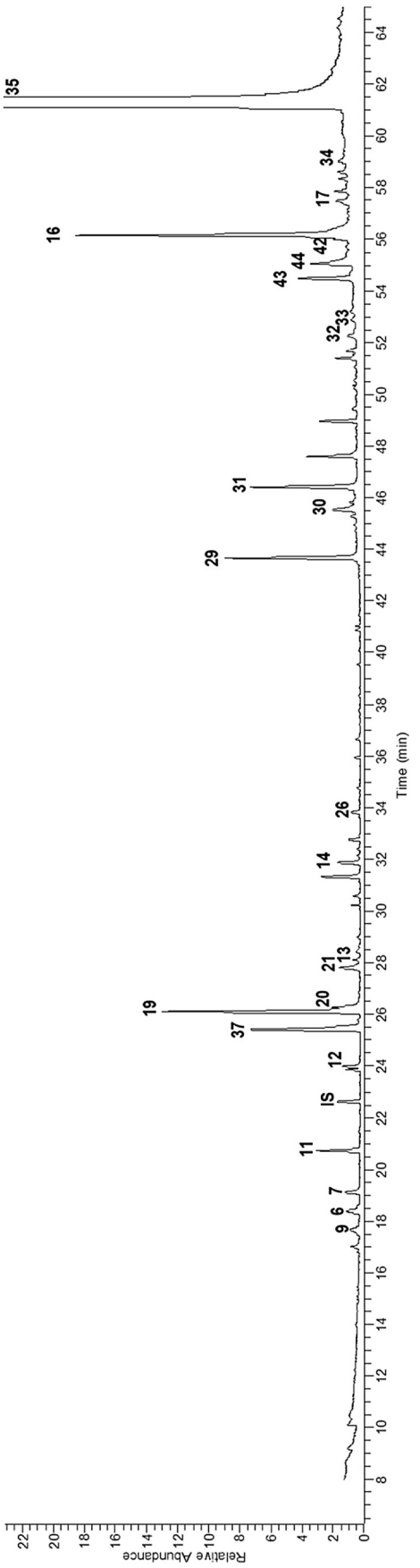

Fig. 1. Typical GC-MS chromatogram (total ion current) of the ethyl acetate extracts of the prepared model wines. Here is presented the corresponding chromatogram of fructose model system submitted to 70 °C for 1 month. For peak identification see Table 3. Only major peaks are highlighted.

Table 2
Relative areas of the volatile organic compounds identified in the 16 model systems at the end of the heating step at 50 °C for 4 months.

KI	Compound	GlcArg	GlcCys	GlcGABA	GlcAsp	FruArg	FruCys	FruGABA	FruAsp
Carbonyls (3)									
1340	Acetol**	0.4 ± 0.0 s	0.4 ± 0.1 s	0.4 ± 0.1 s	0.3 ± 0.1 s	0.9 ± 0.1 ns, ns	0.9 ± 0.1 ns, ns	0.8 ± 0.1 ns, ns	0.8 ± 0.1 s, ns
1640	2-Cyclopentene-1,-4-dione **	n d.	0.4 ± 0.0s	n d.	n d.	1.9 ± 0.1s,s	3.5 ± 0.3 s, s	1.4 ± 0.1 ns, ns	2.7 ± 0.1 s, s
2075	Glycerone **	0.6 ± 0.1 s	0.6 ± 0.0 s	0.5 ± 0.1 s	0.3 ± 0.1 s	1.7 ± 0.0 s, ns	1.2 ± 0.2 ns, ns	1.3 ± 0.1 ns, ns	1.8 ± 0.5 s, ns
Ethyl esters (7)									
1315	Ethyl pyruvate *	1.7 ± 0.3 ns	2.2 ± 0.1 s	2.0 ± 0.1 ns	2.1 ± 0.3 s	1.4 ± 0 .0.1 ns, ns	1.3 ± 0.3 ns, ns	1.1 ± 0.2 s, ns	1.3 ± 0.1 ns, ns
1388	Ethyl lactate *	n d.	n d.	n d.	n d.	1.8 ± 0.0 s, s	1.2 ± 0.1 s, s	1.6 ± 0.0 ns, s	1.8 ± 0.1 s, s
1460	Ethyl glycolate *	0.1 ± 0.0 s	0.3 ± 0.0 s	0.1 ± 0.0 s	0.2 ± 0.0 s	0.9 ± 0.1 ns, s	1.1 ± 0.0. s, s	0.9 ± 0.0 ns, s	1.5 ± 0.1 s, s
1570	Ethyl 3-hydroxybutyrate **	n d.	n d.	n d.	n d.	1.1 ± 0.0 s, s	0.9 ± 0.1 ns, s	1.0 ± 0.0 ns, s	1.3 ± 0.1 s, s
1660	Ethyl levulinate **	n d.	n d.	n d.	n d.	0.3 ± 0.0 s	0.4 ± 0.0 s, ns	0.2 ± 0.0 ns, ns	0.3 ± 0.0 ns, s
2403	Diethyl tartrate *	15.6 ± 0.9 ns	17.0 ± 0.7 ns	11.1 ± 0.6 s	11.4 ± 2.0 s	20.2 ± 0.5 s, s	17.8 ± 1.0 ns, ns	15.2 ± 0.4 ns, ns	19.2 ± 2.0 s, ns
2449	Ethyl hydrogen succinate ***	n d.	n d.	n d.	n d.	1.2 ± 0.1 s, s	0.7 ± 0.1 s, s	1.0 ± 0.1 ns, s	2.0 ± 0.2 s, s
Furans (11)									
1520	Furfural*	n d.	2.1 ± 0.1 s	1.1 ± 0.2 s	n d.	5.7 ± 0.2 s, ns	4.2 ± 0.5 ns, ns	5.80 ± 0.54 s, ns	5.2 ± 0.7 ns, ns
1525	5-Methylfurfural*	n d.	n d.	n d.	n d.	0.7 ± 0.0 s, s	0.7 ± 0.0 s, s	0.59 ± 0.03 ns, ns	0.5 ± 0.0 s, ns
1559	2-Acetylfurfan*	n d.	n d.	n d.	n d.	0.2 ± 0.0 ns, ns	0.2 ± 0.0 ns, ns	0.25 ± 0.03 ns, ns	0.3 ± 0.0 ns, ns
1710	Furfuryl alcohol**	n d.	n d.	n d.	n d.	0.4 ± 0.0 s, s	0.4 ± 0.0 ns, ns	0.36 ± 0.01 ns, ns	0.4 ± 0.1 s, ns
1819	γ-Crotonolactone**	n d.	n d.	n d.	n d.	0.3 ± 0.0 ns, ns	0.4 ± 0.0 s, s	0.26 ± 0.01 ns, ns	0.4 ± 0.0 s, s
1986	5Ethoxymethylfurfural ***	n d.	n d.	n d.	n d.	2.0 ± 0.1 s, s	1.4 ± 0.1 s, s	1.39 ± 0.09 ns, s	1.3 ± 0.1 ns, ns
1979	2,5-Furandi carboxal dehyde **	n d.	n d.	n d.	n d.	6.3 ± 0.1 ns, ns	5.9 ± 0.5 ns, s	5.65 ± 0.21ns, ns	5.8 ± 0.2 ns, ns
2070	Furyl hydroxymethyl ketone **	n d.	n d.	n d.	n d.	3.5 ± 0.1 s, s	3.3 ± 0.2 s, s	2.92 ± 0.11 ns, ns	3.2 ± 0.3 s, ns
2167	5-Acetoxymethylfurfural **	n d.	n d.	0.2 ± 0.0 s	0.2 ± 0.0 s	1.5 ± 0.1 ns, ns	1.4 ± 0.2 s, ns	1.58 ± 0.10 ns, ns	1.1 ± 0.1 s, ns
2498	2-Furoic acid ***	n d.	n d.	n d.	n d.	0.6 ± 0.1 s, s	n d.	n d.	n d.
2589	5-Hydroxymethylfurfural *	16.7 ± 0.9 s	9.1 ± 0.2 s	10.3 ± 0.6 s	8.9 ± 1.0 s	383.4 ± 2.0 s, s	288.3 ± 12.7 ns, s	309.7 ± 8.2 ns, ns	278.8 ± 11.9 s, ns
Organic acids (2)									
1498	Acetic acid *	0.8 ± 0.0 s	7.2 ± 0.7 s	1.0 ± 0.3 s	0.7 ± 0.1 s	3.6 ± 0.1 ns, ns	3.9 ± 0.4 ns, ns	4.1 ± 0.5 s, s	7.0 ± 1.6 s, s
1591	Propionic acid **	n d.	0.4 ± 0.1 s	n d.	n d.	0.3 ± 0.0 s, ns	0.7 ± 0.0 s, s	0.5 ± 0.0 ns, ns	0.9 ± 0.1 s, s
Pyrans (2)									
2287	DDMP ***	n d.	n d.	n d.	n d.	0.8 ± 0.0 s, s	0.6 ± 0.1 ns, ns	0.7 ± 0.0 ns, ns	0.7 ± 0.2 s, ns
2360	5-Hydroxymaltol **	n d.	n d.	n d.	n d.	6.6 ± 0.1 s, s	2.7 ± 0.2 s, ns	5.9 ± 0.3 ns, ns	4.1 ± 0.5 s, s
	Number of Compounds	7	10	9	8	25	24	24	24

KI	Compounds	FruGlc(4aa)	Glc	Fru	FruGlc	FruArg(x2)	FruCys(x2)	FruGABA(x2)	FruAsp(x2)
Carbonyls (3)									
1340	Acetol**	0.5 ± 0.1 ns	0.3 ± 0.0s	0.7 ± 0.0	0.6 ± 0.0 ns	0.9 ± 0.2 ns	0.6 ± 0.1 ns	0.8 ± 0.2 ns	0.7 ± 0.1ns
1640	2-Cyclopentene-1,-4-dione **	2.0 ± 0.0 s	n d.	1.5 ± 0.1	0.6 0.0s	1.4 ± 0.1 ns	2.8 ± 0.1 s	1.3 ± 0.1 ns	2.3 ± 0.2 s
2075	Glycerone **	0.8 ± 0.0 ns	n d.	1.2 ± 0.1	0.9 ± 0.1 ns	1.4 ± 0.3 ns	0.9 ± 0.1 ns	1.2 ± 0.2 ns	1.4 ± 0.1 ns
Ethyl esters (7)									
1315	Ethyl pyruvate *	1.2 ± 0.2 ns	1.8 ± 0.2 ns	1.6 ± 0.1	1.6 ± 0.1 ns	1.5 ± 0.3 ns	1.1 ± 0.2 ns	1.0 ± 0.1 s	1.3 ± 0.2 ns
1388	Ethyl lactate *	0.8 ± 0.0. s	n d.	1.5 ± 0.1	0.9 ± 0.0 s	1.5 ± 0.0 s	0.9 ± 0.0 s	1.3 ± 0.1s	1.3 ± 0.1 s
1460	Ethyl glycolate *	0.7 ± 0.0s	n d.	0.9 ± 0.0	0.6 ± 0.0 s	0.73 ± 0.0 s	0.8 ± 0.1 ns	0.7 ± 0.1 s	0.8 ± 0.0 s
1570	Ethyl 3-hydroxybutyrate **	0.6 ± 0.0 s	n d.	0.9 ± 0.1	0.5 ± 0.0s	0.9 ± 0.0 ns	0.6 ± 0.1 s	0.8 ± 0.1 ns	0.8 ± 0.1 s
1660	Ethyl levulinate **	0.2 ± 0.0 s	n d.	0.3 ± 0.0	n d.	0.2 ± 0.0 ns	0.4 ± 0.0s	0.2 ± 0.0 s	0.2 ± 0.0 ns
2403	Diethyl tartrate *	15.1 ± 0.6 ns	11.6 ± 0.4 s	15.3 ± 0.8	14.4 ± 1.1 ns	15.1 ± 0.7 ns	16.0 ± 0.7 ns	13.0 ± 0.8 ns	16.3 ± 0.9 ns
2449	Ethyl hydrogen succinate ***	0.7 ± 0.1 s	n d.	1.0 ± 0.2	0.5 ± 0.1 s	0.7 ± 0.1 s	0.5 ± 0.1 s	0.8 ± 0.1 s	1.1 ± 0.1 s
Furans (11)									
1520	Furfural*	3.7 ± 0.2 s	n d.	4.7 ± 0.5	4.1 ± 0.3 ns	4.6 ± 0.4 ns	3.5 ± 0.7 s	5.4 ± 0.5 ns	4.9 ± 0.2 ns
1525	5-Methylfurfural*	0.4 ± 0.0 s	n d.	0.6 ± 0.0	0.4 ± 0.6 s	0.6 ± 0.0 ns	0.5 ± 0.0 s	0.5 ± 0.1 s	0.5 ± 0.1 s
1559	2-Acetylfurfan*	0.2 ± 0.0 s	n d.	0.3 ± 0.1	n d.	0.2 ± 0.0 ns	0.2 ± 0.0 s	0.3 ± 0.0 ns	0.3 ± 0.0 ns
1710	Furfuryl alcohol**	0.2 ± 0.1s	n d.	0.3 ± 0.0	0.2 ± 0.1 s	0.3 ± 0.0 ns	0.3 ± 0.0 ns	0.3 ± 0.0 ns	0.4 ± 0.0 ns
1819	γ-Crotonolactone**	0.3 ± 0.0 s	n d.	0.3 ± 0.0	0.2 ± 0.0 s	0.3 ± 0.0 ns	0.3 ± 0.0 s	0.2 ± 0.0 ns	0.3 ± 0.0 ns
1986	5Ethoxymethylfurfural ***	0.6 ± 0.0 s	n d.	1.3 ± 0.1	0.6 ± 0.1 s	1.5 ± 0.1 s	1.0 ± 0.0 s	1.2 ± 0.0 s	1.2 ± 0.0 s
1979	2,5-Furandi carboxal dehyde **	3.5 ± 0.2 s	n d.	6.1 ± 0.2	3.5 ± 0.2 s	5.8 ± 0.3 ns	4.4 ± 0.2 s	5.2 ± 0.3 s	5.2 ± 0.3 s
2070	Furyl hydroxymethyl ketone **	1.5 ± 0.1 s	n d.	2.8 ± 0.2	1.4 ± 0.1 s	2.8 ± 0.1 ns	2.9 ± 0.1 ns	2.7 ± 0.2 ns	2.9 ± 0.1 ns
2167	5-Acetoxymethylfurfural **	1.0 ± 0.1 s	n d.	1.6 ± 0.1	1.2 ± 0.1s	1.8 ± 0.1 ns	1.2 ± 0.1 s	1.5 ± 0.1 ns	1.2 ± 0.1 s
2498	2-Furoic acid ***	n d.	n d.	n d.	n d.	n d.	n d.	n d.	0.4 ± 0.0 s
2589	5-Hydroxymethylfurfural *	153.5 ± 2.4 s	3.9 ± 0.1 s	298.4 12.6	178.4 ± 10.1 s	303.2 ± 3.8 ns	227.3 ± 7.9 s	289.5 ± 15.9 ns	272.1 ± 15.9 s
Organic acids (2)									
1498	Acetic acid *	3.1 ± 0.3 ns	0.6 ± 0.1 s	2.7 ± 0.4	2.0 ± 0.ns	3.6 ± 0.2 ns	4.4 ± 0.2 s	3.7 ± 0.8 ns	3.5 ± 0.2 ns
1591	Propionic acid **	0.6 ± 0.0 s	n d.	0.5 ± 0.1	0.2 ± 0.0 s	0.4 ± 0.1 ns	0.5 ± 0.0 ns	0.4 ± 0.1 ns	0.5 ± 0.0 ns
Pyrans (2)									
2287	DDMP ***	0.3 ± 0.1 s	n d.	0.6 ± 0.1	0.4 ± 0.0 s	0.6 ± 0.1 ns	0.5 ± 0.0 ns	0.7 ± 0.1 ns	0.6 ± 0.0 ns
2360	5-Hydroxymaltol **	1.7 ± 0.2 s	n d.	5.3 ± 0.5	2.1 ± 0.2 s	5.0 ± 0.6 ns	2.5 ± 0.3 s	5.8 ± 0.5 ns	5.0 ± 0.2 ns
	Number of Compounds	24	5	24	22	24	24	24	25

Mean value (n = 6) ± standard deviation; n d. – not detected; s – significant difference($p < 0.050$); ns –no significant difference($p > 0.050$). First letter compares with Fru model system and the second with the corresponding model system when the amino acid concentration was duplicated.
DDMP - 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one.
*MS data and Kovats index in agreement with those of authentic compound; **MS data and Kovats index in agreement with those in literature; ***MS data in agreement with those in NIST08 and Wiley 6.0 libraries.

Table 3
Relative areas of the volatile organic compounds identified in the model systems submitted to 70 °C for 1 month.

#	KI	Compound	Common odour descriptors [a]	Fru	FruCys	Glc
	Acetals (2)					
1	1508	Glycolaldehyde diethyl acetal ***		1.3 ± 0.2	0.8 ± 0.2 s	n d.
2	1513	Furfural diethyl acetal **	Fruity, earthy, mushroom	0.2 ± 0.0	0.4 ± 0.1 s	n d.
	Alcohols (2)					
3	1851	Butoxyethoxyethanol **		0.1 ± 0.0	0.2 ± 0.0 s	0.2 ± 0.0 ns
4	2380	2,6-Di-*tert*-butyl phenol **		n d.	n d.	0.5 ± 0.1 s
	Carbonyls (4)					
5	1100	2,3-Pentadione **	Caramel, sweet, fruity, buttery, fresh	0.5 ± 0.1	0.9 ± 0.1 s	n d.
6	1338	Acetoin **	Fatty, buttery	1.0 ± 0.1	0.5 ± 0.1 s	n d.
7	1355	Acetol **		1.0 ± 0.2	1.0 ± 0.1 ns	n d.
8	1649	2-Cyclopentene-1,4-dione **		0.1 ± 0.0	0.2 ± 0.0 s	n d.
	Ethyl esters (9)					
9	1315	Ethyl pyruvate *	Ethereal, fruity, sweet, vegetable, caramel	1.0 ± 0.1	0.8 ± 0.2 ns	0.4 ± 0.1 s
10	1380	Ethyl 3-ethoxypropionate*		0.1 ± 0.03	0.3 ± 0.1 s	n d.
11	1388	Ethyl lactate*		2.1 ± 0.2	1.8 ± 0.1 ns	0.3 ± 0.0 s
12	1460	Ethyl glycolate*		0.9 ± 0.1	0.4 ± 0.1 s	0.5 ± 0.1 s
13	1570	Ethyl 3-hydroxybutyrate **		0.4 0.0	0.5 ± 0.0 s	0.2 ± 0.1 s
14	1660	Ethyl levulinate **		1.3 ± 0.1	0.5 ± 0.0 s	0.2 ± 0.0 s
15	1731	Diethyl succinate*	Fabric, fruity, watermelon, flower, sweaty,potato	0.2 ± 0.0	0.3 ± 0.0 s	0.2 ± 0.0 ns
16	2403	Diethyl tartrate*		16.8 ± 1.9	13.9 ± 1.3 s	12.4 ± 0.7 s
17	2449	Ethyl hydrogen succinate***		0.5 ± 0.1	2.0 ± 0.4 s	1.1 ± 0.1 s
	Furans (12)					
18	1341	2,5-Diethoxytetrahydrofuran***		0.2 ± 0.0	n d.	n d.
19	1522	Furfural*	Woody, almond, sweet, fruity, flowery	10.2 ± 0.9	7.4 ± 0.6 s	1.0 ± 0.1 s
20	1526	5-Methylfurfural*	caramel, burnt sugar, spicy, acid, coffee	1.6 ± 0.2	1.1 ± 0.2 s	n d.
21	1565	2-Acetylfuran *	Balsamic, cinnamon, sweet, cereal	1.5 ± 0.1	1.3 ± 0.2 s	n d.
22	1579	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furanone***		0.3 ± 0.0	0.5 ± 0.1 s	n d.
23	1674	2-Acety-5-methyl furan **	Nutty, hay, caramel, coumarin	0.2 ± 0.0	0.4 ± 0.1 s	n d.
24	1680	Ethyl 2-furoate*		0.2 ± 0.0	0.4 ± 0.1 s	n d.
25	1689	5,5-dimethyl-2(5H)-furanone **		0.6 ± 0.1	0.4 ± 0.1 s	n d.
26	1717	Furfuryl alcohol **	Fermented, burnt sugar, creamy, caramel	0.6 ± 0.1	0.6 ± 0.0 ns	0.2 ± 0.0 s
27	1744	β-Angelica lactone **		0.2 ± 0.0	0.3 ± 0.1 s	n d.
28	1825	γ-Crotonolactone **		0.2 ± 0.0	0.4 ± 0.1 s	n d.
29	1995	5-Ethoxymethylfurfural **		7.0 ± 0.7	6.0 ± 0.4 s	0.2 ± 0.0 s
30	2053	2,5-Furandicarboxaldehyde **		2.6 ± 0.6	3.0 ± 0.5 ns	0.5 ± 0.1 s
31	2080	Furyl hydroxymethyl ketone **		6.0 ± 0.6	5.5 ± 0.5 ns	n d.
32	2269	Sotolon *	Spicy, mushroom, curry, burnt, maple	0.7 ± 0.1	1.0 ± 0.1 s	n d.
33	2289	Abhexone ***	Sweet, fruity, with a brown maple note	0.4 ± 0.1	1.0 ± 0.1 s	n d.
34	2506	2-Furoic acid ***		0.7 ± 0.1	2.2 ± 0.4 s	n d.
35	2592	5-Hydroxymethyfurfural **	Fatty, musty, wax flowers	306.4 ± 46.4	208.6 ± 18.5 s	12.9 ± 0.8 s
36	2689	(S)-3-Hydroxy −(γ)-Butyrolactone ***		0.5 ± 0.1	2.1 ± 0.1 s	n d.
	Organic acids (6)					
37	1503	Acetic acid*	Sour, vinegar, pungent	5.5 ± 0.2	4.7 ± 0.3 s	3.6 ± 0.2 s
38	1572	Formic acid **	Pungent, vinegar, formyl	n d.	n d.	0.4 ± 0.0 s
39	1594	Propionic acid**		0.2 ± 0.0	0.4 ± 0.1 s	0.3 ± 0.1 ns
40	1721	Isovaleric acid*	Sweaty, cheesy, rancid	0.1 ± 0.0	0.3 ± 0.1 s	0.3 ± 0.0 s
41	2121	Octanoic acid*	Fatty, cheesy, fresh, moss	n d.	n d.	0.2 ± 0.0 s
42	2398	Levulinic acid**	Sweet, caramel, acid, buttery	1.0 ± 0.2	1.2 ± 0.2 ns	n d.
	Pyrans (2)					
43	2345	Hydroxydihydromaltol **		3.2 ± 0.5	2.7 ± 0.4 ns	n d.
44	2365	5-Hydroxymaltol**	Roasted	3.8 ± 0.3	3.1 ± 0.4 s	n d.
	Number of Compounds			41	40	20

* MS data and Kovats index in agreement with those of authentic compound; ** MS data and Kovats index in agreement with those in literature; *** MS data in agreement with those in NIST08 and Wiley 6.0 libraries.

[a] Based on online databases: The Pherobase (www.pherobase.com/) and The Good Scents Company Information System (http://www.thegoodscentscompany.com/). Values are means of triplicates (n = 6) ± SD; n.d. - not detected; s - significant difference when compared with Fru MS ($p < 0.050$); ns - no significant difference when compared with Fru MS ($p > 0.050$).

molecules, originating furanic derivatives, essentially HMF (from a 1,2-endiol) (Belitz et al., 2009, pp. 248–339). Additionally, the dehydration of Amadori/Heyns compounds via 1,2-enolization might have contributed for the formation of furans in the model systems wherein the amino acids were present. Indeed, the 1,2-enolization is favored by low pH instead the 2,3-enolization (Parker, 2015). The 2,3-enolization originates carbonyl compounds, namely furanones and pyranones (Mottram, 2007; Parker, 2015). Despite being less favored in acidic medium, the 2,3-enolization has occurred, although in lower extension, since that 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) and acetol, typical markers of this pathway (Davidek, Clety, Devaud, Robert, & Blank, 2003; Martins, Jongen, & van Boekel, 2000), were identified. DDMP was only found in model systems containing Fru and acetol was found in all model systems submitted to 50 °C for 4 months. However, DDMP was not identified in those model systems that were overheated. Other furan identified was 5-ethoxymethylfurfural. Cutzach, Chatonnet, and Dubourdieu (1999) found that it is formed during the ageing process of sweet fortified wines and suggested that its presence can be justified by the reaction between ethanol and HMF. This furan was abundantly found in sweet Madeira wines submitted to 70 °C (V. Pereira et al., 2014) and has been identified in wood-aged Madeira wines (Câmara, Alves, & Marques, 2006).

Sotolon (4,5-dimethyl-3-hydroxy-2(5)H-furanone) is commonly considered as being a key odorant of sweet fortified wines that are submitted to oxidative ageing (Câmara, Marques, Alves, & Silva Ferreira, 2004; Collin, Nizet, Bouuaert, & Despatures, 2012; Silva Ferreira, Barbe, & Bertrand, 2003), imparting a powerful odor of spicy/curry notes, not only in Madeira but also in Porto, Sherry and Vins doux Naturels. Conversely, its occurrence in white wines is considered detrimental for the fresh taste and bouquet, and for that reason it has been reported as a chemical marker of the oxidative wine spoilage (Pons et al., 2010). Despite its significance, the formation mechanism of sotolon in wine is not consensual so far. However, Maillard reaction has been pointed out by many authors as being one of the sotolon formation route (Guerra & Yaylayan, 2011; Hofmann & Schieberle, 1997; Oliveira e Silva et al., 2008). This study indicates that sotolon can be generated from Fru degradation in acidic medium favored by high temperatures, since it was only identified in model systems exclusively prepared with Fru in synthetic wine, reducing the contribution of Maillard reaction in its formation. Other 2(5H)-furanones were also identified, namely 5,5-dimethyl-2(5H)-furanone, β-angelica lactone, γ-crotonolactone and abhexone. The occurrence of angelica lactones in wines has been associated with sugar degradation (Pisarnitskii, 2001), conferring sweet, creamy, coconut and vanilla nuances (van Ruth, 2003). Actually, it was confirmed that angelica lactone is a Fru by-product due to thermal degradation in acid medium. Abhexone, which has a powerful aroma reminiscent of maple syrup, has been reported as playing a significant role in food flavor due to its extremely low odor threshold values (0.01 parts per trillion (ppt)), even lower than sotolon (1 ppt) (Nakahashi, Yaguchi, Miura, Emura, & Monde, 2011).

The identified group of ethyl esters may result from the organic acid esterification in the presence of ethanol, namely the ethyl esters from pyruvic acid, lactic acid, glycolic acid and levulinic acid.

Finally, organic acids were also found in the current model systems, and their formation can follow the scheme suggested by Ginz, Balzer, Bradbury, and Maier (2000). Actually, formic acid and acetic acid have been frequently found in Glc and Fru model systems (Ginz et al., 2000; Martins et al., 2000). Acetic acid, propionic acid and formic acid were also found.

4. Conclusions

Fru model systems develop more browning, antioxidant activity and VOCs than Glc model systems, due to Fru being more reactive than Glc. Sugar derived-products are likely to represent up to 20–30% of the browning developed by sweet Madeira wines and about a third in terms of antioxidant activity. Considering that an important number of VOCs (31/47) formed in the current model systems were already identified in Madeira wines submitted to *estufagem*, it can be concluded that their occurrence in Madeira wine might be derived from sugar degradation, mostly from Fru. These sugar derived-products belong to different chemical families, such as acetals, alcohols, carbonyls, ethyl esters, organic acids, pyrans and furans. Furans are the major chemical family formed, namely HMF, which represents about 88% of the Fru model systems VOCs. The prepared model systems showed that VOC profile is independent from amino acids, but strongly dependent from the sugar type. The formation of Maillard reaction VOCs could not be evidenced under the Madeira sweet wine conditions, due to the high sugar content. However, this does not mean that Maillard reaction does not occur, since that model systems containing amino acids in general developed more browning. For the first time, it could be concluded that sotolon formation in Madeira-type wines can also be derived from Fru degradation in acidic medium, namely at 70 °C. This reduces the contribution of Maillard reaction to explain its formation. Nevertheless, the amino acids (namely Cys) seem to have a greater impact in the browning formation.

For all these reasons it can be concluded that Fru degradation mechanisms have a greater contribution in the development of the typical features of sweet fortified wines than those of Glc, namely in terms of color and aroma.

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