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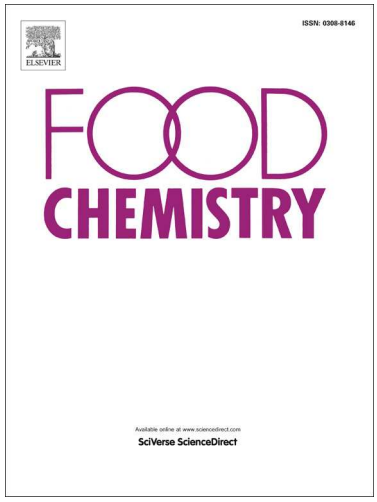

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The effect of ageing temperature on the physicochemical properties, phytochemical profile and α-glucosidase inhibition of *Hibiscus sabdariffa* (roselle) wine

Idolo Ifie[1], László Abrankó [2], Jose A Villa-Rodriguez[1], Nóra Papp [2], Peter Ho[1], Gary Williamson[1] and Lisa J. Marshall[1]*

[1]School of Food Science and Nutrition, University of Leeds, Leeds LS2 9JT, United Kingdom

[2]Szent István University, Faculty of Food Science, Department of Applied Chemistry, 29-43 Villányi, Budapest, H-1118, Hungary

*Corresponding author L.J.Marshall@leeds.ac.uk Tel +44(0)1133431952

Abstract

The effect of temperature (6, 15 and 30 °C) during ageing on the colour, phytochemical composition and bioactivity of roselle wine was investigated over 12 months. At the end of aging, wines stored at 6 °C had the highest colour density and lowest polymeric anthocyanins. The initial concentration of most of the individual phenolic compounds decreased during ageing, with reduction of monomeric anthocyanins contributing to the formation of anthocyanin-derivatives (pyranoanthocyanins), eight of which were identified tentatively and reported here for the first time in roselle wine. The decrease in individual phenolic compounds did not affect inhibition of α-glucosidase (maltase) activity, which remained relatively low but stable throughout ageing. Diethyl succinate was the only volatile clearly influenced by ageing temperature, with the most pronounced effect at 30 °C (~ 256 fold increase). In summary, the final concentrations of anthocyanins and diethyl succinate were the major compounds influenced by ageing temperature.

Keywords: *Hibiscus sabdariffa*; roselle wine; ageing temperature; phytochemicals; pyranoanthocyanins; α-glucosidase inhibition.

1. Introduction

H. sabdariffa (roselle) has emerged as a competitive target for the development of functional foods due to its high content of bioactive phytochemicals with properties that may reduce the risk of diseases, such as hypertension (Odigie, Ettarh & Adigun, 2003), obesity (Alarcon-Aguilaret al., 2007) and diabetes (Peng, Chyau, Chan, Chan, Wang & Huang, 2011). *H. sabdariffa* calyces and their extracts are currently being used either as a whole or as bioactive ingredients in the production of refreshing drinks, jams, jellies, flour for biscuits, yogurts, pizza, wine etc (Da-Costa-Rocha, Bonnlaender, Sievers, Pischel & Heinrich, 2014). These processing techniques can impact greatly the content and the bioactive properties of *H. sabdariffa;* therefore it is important to investigate the magnitude that processing conditions have on the final product.

Recently, the impact of fermentation temperature (20 and 30 °C) on the phytochemical profile and bioactivity of *H. sabdariffa* wine (roselle wine) was investigated showing that fermentation at 20 °C produced more fruity aromas and was slightly more active in the inhibition of α-glucosidase (Ifie, Marshall, Ho & Williamson, 2016). However, young wine requires ageing (maturation) and the final quality of wine is the result of multiple interactions between all the chemical components and specific environmental factors such as the ageing temperature (Styger, Prior & Bauer, 2011). The research until date on roselle wine during maturation have tended to focus on the physicochemical changes that occur during the process (Mounigan & Badrie, 2007), but to the best of our knowledge, the transformation in the phytochemical profile and the subsequent effect on the bioactivity of *H. sabdariffa* (inhibition of α-glucosidase) during long term storage is yet to be reported. This information is needed in order to establish quality control measures and provide valuable information on the more suitable practices during ageing process.

The changes in the phenolic compounds in red wines during the ageing process are very complex and decreases in flavonoids and anthocyanins content are commonly observed with the rate of degradation being more pronounced with the anthocyanins (Sun & Spranger, 2005). This decrease in the concentration of monomeric anthocyanins occurs because they progressively react with other wine components leading to the formation of more complex and stable anthocyanin-derived pigments including pyranoanthocyanins and polymeric anthocyanins produced from condensation between anthocyanin and/or flavan-3-ols directly or mediated by aldehydes (Somers & Pocock, 2015).

During ageing, wine acquires aromatic complexity resulting from different phenomena such as: esterification/hydrolysis reactions, redox reactions, spontaneous clarification and CO_2 elimination. This results in the loss of the characteristics aromas linked to the grape variety (or the raw material), and modifications of the volatiles formed during alcoholic fermentation, which ultimately leads to the formation of new aromas characteristic of older wines (Câmara, Alves & Marques, 2006). However, in roselle wine, there is no information on the changes in volatile composition during ageing.

Some of the reported modifications in volatile composition include the reduction in concentrations of fusel alcohol acetates and ethyl esters of straight–chain medium fatty acids, while the levels of the branched chain fatty acid ethyl esters are more or less stable or even increase during aging (Sumby, Grbin & Jiranek, 2010). In the case of higher alcohols, the changes that occur are not clear, while some researchers have reported increases in the concentration of higher alcohols, the reduction in specific alcohols has also been observed in some published studies (Câmara et al., 2006). These compounds (higher alcohols) account for about 50 % of aroma compounds, with isoamyl alcohol (fusel note) and 2-phenyl ethanol (rose and flowery note) being major contributors to the global aroma of the final product.

Since ageing conditions contribute significantly to overall wine quality, this study was undertaken to investigate the impact of ageing temperature on roselle wine colour, phenolic profile and inhibition of α-glucosidase potential as well as stability of the aroma compounds.

2. Materials and methods

2.1. Materials and chemicals

Commercial standards of delphinidin 3-*O*-sambubioside (DS), cyanidin 3-*O*-sambubioside (CS) and quercetin, were purchased from Extrasynthese, Genay, (France), while trifluoroacetic acid, LC-MS grade formic acid, gallic acid, caffeic acid, Folin-Ciocalteu's reagent and ethanol were from Sigma Aldrich, St Louis, Missouri, (USA). 2-phenylethanol, isoamyl alcohol, ethyl acetate, ethyl hexanoate, ethyl decanoate, ethyl lactate, isoamyl acetate, methyl butyrate, intestinal acetone powder from rat, glucose assay reagent, maltose and sodium mono and dibasic phosphate were from Sigma-Aldrich, Dorset, (UK); while 1-hexanol, eugenol, diethyl succinate and ethyl octanoate were from Alfa Aesar, Lancashire, (UK). Sodium carbonate was from Fisher scientific, Loughborough (UK) and the solid phase extraction Oasis MAX cartridge 3 mL (60 mg) was purchased from Waters Corporation Ltd., Massachusetts (USA). All other standards used in the study were either analytical grade, HPLC or GC grade.

2.2. Wine ageing/storage conditions

Roselle wine was produced from *H. sabdariffa* calyces by fermenting the dark red calyces at 20 °C as described in Ifie et al. (2016). The oenological parameters of the wine at bottling were pH 3.0, soluble solids 2 g/L, total acidity 5.74 g/L, volatile acidity (measured as acetic acid) 0.30 g/L, alcohol content 11.5 % v/v and ash content 4.8 g/L. Three temperature conditions 6 °C, (low temperature) 15 °C (control) and 30 °C (high temperature) were chosen

for ageing of wine samples that lasted for 12 months. Duplicate bottles were prepared for each ageing temperature, bringing the total number of bottles used in the experiment to 6 and the bottles were placed in slanting positions in incubators (Sanyo-MIR-153, Japan) set at the experimental ageing temperatures.

2.3. Wine analysis during ageing

Colour measurements were evaluated on months 2, 4, 8 and 12. The changes in phenolic compounds were monitored on months 1, 2, 3, 4, 6, 8 and 12, while the effect of ageing conditions on total phenolic content and α-glucosidase inhibition was evaluated on months 0, 2, 4, 8, and 12. Roselle wine volatiles were analysed at the end of the ageing period. Wine samples (5 mL) were taken from each bottle kept at the ageing temperature and the analysis were all performed in triplicates (n = 6).

2.3.1. Colour analysis

Colour density (CD), hue tint (HT) and polymeric anthocyanins (PA) were measured according to the method of Giusti &Wrolstad (2001) using a spectrophotometer (CECIL CE 3021 Series). For the analysis of polymeric anthocyanins, 0.2 mL of 20 % potassium metabisulphite ($K_2S_2O_5$) was added to 2.8 mL of wine sample and the mixture allowed to equilibrate for 15 min. Thereafter, an aliquot of sample (200 µL) was placed in a 1 mm pathlength cuvette and the colour indices were determined as follows:

$$CD = [(A_{420\ nm} - A_{700\ nm}) + (A_{520\ nm} - A_{700\ nm})] \quad (1)$$

$$HT = [(A_{420\ nm} - A_{700\ nm}) / (A_{520\ nm} - A_{700\ nm})] \quad (2)$$

$$\%\ PA = \underline{[(A_{420\ nm} - A_{700\ nm}) + (A_{520\ nm} - A_{700\ nm})]\ SO_2}/CD \times 100 \quad (3)$$

2.3.2. Analysis of phenolic compounds

The identification and quantification of phenolic compounds in roselle wine was carried out as described in Ifie et al. (2016). A UFLCXR system (Shimadzu) consisting of a binary pump, coupled with an online unit degasser (DGU-20A3/A5), a solvent delivery module (LC-20AD), a thermostat autosampler/injector unit (SIL-20A) and a photodiode array with multiple wavelength (SPD-20A) was used for the analysis. The mobile phase was 0.1 % (v/v) trifluoroacetic acid (mobile phase A) and trifluoroacetic acid/acetonitrile/water (50:49.9:0.1) (mobile phase B). The flow rate was set to 1 mL/min and the injection volume was 10 µL. Chromatographic separation was performed on a Gemini C18 5µm, 250 mm ×4.6 mm column (Phenomenex, Macclesfield, UK) and the oven temperature was maintained at 35 °C. The photodiode array detector was set to measure at wavelengths of 265, 320, 360 and 520 nm. The gradient program was started at 8 % B and increased linearly until 18 % solvent B was achieved at 3.50 min, solvent B was increased to 32 % at 18 min, 60 % at 28 min, and then 100 % at 32 min. Finally, 100 % B was kept constant for 4 min, followed by 3.5 min isocratic re-equilibration for initial conditions.

2.3.3. Identification of anthocyanins derivatives in roselle wine

The HPLC system (Agilent 1200, Agilent Technologies, Waldbronn, Germany) including a binary pump and a diode array detector (DAD) was coupled to an Agilent 6350 quadrupole – time-of-flight (Q/TOF) hybrid tandem mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) equipped with a dual-spray ESI source. Chromatographic separation was carried out on a Kinetex C18 4.6×150mm, 2.6-µm particle size column (Phenonemex, Macclesfield, UK), using 0.5% (v/v) formic acid in water (mobile phase A) and 0.5% (v/v) formic acid in acetonitrile (mobile phase B) as mobile phases at a flow rate of 500 µL/min. The gradient program was started at 10% B and after 5 min of isocratic run, solvent B was increased linearly and reached 35% at 45 min and then 100% at 45 min. Finally, 100% B was kept

constant for 5min, followed by 15 min isocratic re-equilibration for initial conditions. The DAD was recording signals at 420 nm and 520 nm, and in all cases 700 nm was used as the reference wavelength. Mass spectrometer was used in positive ion mode, with the following parameters: electrospray capillary voltage, 4000 V; nebulizer pressure, 40 psig; drying gas flow rate, 13 L/min; gas temperature, 350 °C; skimmer voltage, 65 V; 160 V fragmentor voltage (FV) The instrument performed continuous automatic internal mass calibration automatically using reference masses according to the manufacturers recommendation. High resolution (>20 000 FWHM at *m/z* 922) full scan TOF spectra were recorded in the range of *m/z* 50–1100, whereas targeted Q/TOF product ion spectra of previously selected precursor masses were acquired using high purity (6.0) N_2 as collision gas and collision energy (CE) was set individually to each compound as a function of precursor *m/z* according to the following function: CE = 0.015 V × *m/z* + 8V. Agilent Mass Hunter Qualitative Analysis Software (version B.06.00 build 6.0.633.0) was applied for data evaluation.

2.3.4. Total phenolic content

Total phenolic content was evaluated using the Folin assay method of Singleton & Rossi (1965) with some modifications. The assay contained the sample (1 mL) with 80 % methanol solution (1:10), 5 mL of diluted Folin-Ciocalteu's phenol reagent (1:10) and 4 mL of 75 g/L sodium carbonate solution. The mixture was then transferred to a water bath (GLS Aqua 12 plus) set at 26 °C and the absorbance reading measured at 765 nm with a spectrophotometer (Cecil 3000 series) after 2 h. The quantification of phenolic content was performed using gallic acid as standard.

2.3.5. Analysis of volatile compounds in roselle wine

2.3.5.1 Extraction of headspace volatiles

The extraction of headspace volatiles was performed with an automated SPME device equipped with a 65 μm PDMS/DVB fibre (Supelco, Bellefonte, PA). An aliquot of wine sample (10 mL), 2-octanol as internal standard (10 μL) and 1.5 g of NaCl were blended together in a 20 mL magnetic crimp sample vial. The SPME fibre was introduced into the injector port after the sample had been equilibrated for 30 min at 40 °C with agitation on and off at 30 s intervals.

2.3.5.2 GC-MS analysis

The GC-MS used for the analysis was a GC (Varian 3800) equipped with an MS detector (Saturn 2200), and a CTC CombiPAL autosampler (CTC analytics, Zwingen, Switzerland). The separation was performed on a Phenomenex ZB-WAX column (30 m × 0.32 mm × 0.50 μm film thickness). Helium was the carrier gas at a flow rate of 1.0 mL/min and the injector temperature was set at 250 °C. The oven program was as follows: 40 °C for 10 min, increased to 100 °C at 15 °C/min, and then to 250 °C at 15 °C/min and held at 250 °C for 5 min. The MS was operated in electron ionization mode (70 eV), and scanning was programmed for a m/z range of 29−300. Identification of volatile compounds was achieved by comparison with commercial standards, matched spectra and fragmentation patterns for compounds reported in the literature. For quantitation of volatiles, stock solutions of standards were dissolved in dichloromethane, and thereafter, working concentrations (up to 6 levels) were prepared by diluting to appropriate levels in a synthetic wine solution (pH adjusted to 3.0 with NaOH) containing 10% ethanol and 3.0 g/L malic acid.

2.3.6. Inhibition of α-glucosidase

Prior to conducting the experiment, residual sugars in wine samples were removed by SPE to prevent any interference and the enzymatic assay was conducted as previously reported (Ifie

et al., 2016). The assay contained 200 µL of substrate (maltose, final concentration of 3 mM) and enzyme, 50 µL sodium phosphate buffer (10 mM pH 7.0) and 50 µL of the inhibitor (roselle wine extract). For the control assay, the inhibitor was replaced by an equal volume of the buffer. The enzyme source was an acetone protein extract from rat intestine (10 mg/mL), prepared in sodium phosphate buffer (10 mM pH 7.0) to give a concentration of 4 mg/mL in the assay. Before introducing the enzyme to the assay solution to initiate the reaction, the enzyme stock solution and the assay mixture containing the inhibitor, buffer and substrate were pre-incubated at 37 °C in a water bath for 10 min. The reaction was carried out at 37 °C for 20 min and stopped by placing the sample in a water bath at 100 °C for 10 min. Polyphenols were removed from the sample by SPE, carried out using Oasis MAX cartridges before adding the hexokinase reagent. The resulting sample (50 µL) was added to 250 µL of hexokinase reagent, placed in a 96 well plate and the absorbance recorded at 340 nm. The rate of enzyme inhibition was calculated as a percentage of the control (without inhibitor).

2.3.7. Statistical analysis

Statistical analysis was performed by one-way analysis of variance using the Statistical Analysis System (SAS) version 9.4 software and significant differences were assessed with the least significant difference (LSD) test ($p \leq 0.05$).

3. Results and discussion

3.1. Wine colour during ageing

The temperature of ageing plays a vital role in wine pigment degradation and polymerisation and it is a primary environmental factor that influences changes in the colour characteristics of red wine (Gómez-Plaza, Gil-Muñoz, López-Roca & Martínez, 2000; Somers et al., 2015). Furthermore, the duration of storage also influences wine colour since most of the changes

occurring during storage are time dependent (Dallas & Laureano, 1994). These two factors together with the pH, SO_2 and alcohol content affect the physicochemical equilibria and structure of wine pigments and are responsible for subtle alteration in hue angle or in hue tint during wine ageing (Dallas et al., 1994).

In this experiment, there was a steady increase in hue and polymeric anthocyanins (PA) colour readings as ageing progressed with wines stored at 30 °C having the highest measurements (Table 1). The increase in these colour indices occurred concurrently with a steady decline in colour density (CD) and at the end of ageing, there was a significant difference ($p \leq 0.05$) between the initial (after fermentation) and final readings for all ageing conditions and also between ageing temperatures. This is consistent with results obtained by other researchers who have reported the steady increase in PA and hue tint during ageing of wines (Dallas et al., 1994; Gómez-Plaza et al., 2000). The decrease in CD as demonstrated in this experiment was also observed in two separate studies with grape wines stored for 12 and 24 months as well as in raspberry wines aged for 6 months (Castillo-Sánchez, García-Falcón, Garrido, Martínez-Carballo, Martins-Dias & Mejuto, 2008; . Gómez-Plaza, Gil-Muñoz, López-Roca, Martínez-Cutillas & Fernández-Fernández, 2002; Rommel, Heatherbell & Wrolstad, 1990). In contrast to our observation, CD remained relatively constant during ageing of grape wines stored at 15 °C for 12 months, despite the reduction in monomeric anthocyanins (García-Falcón, Pérez-Lamela, Martínez-Carballo & Simal-Gándara, 2007). In such instances, it has been suggested that the monomeric anthocyanins are all polymerised (rather than lost through degradation) and these polymerised anthocyanins contribute to the colour of wine. Furthermore, the formation of reddish brown pigments during ageing that absorb at 420 nm add to the colour density ($A_{420\ nm} + A_{520\ nm}$) and might offset any reduction in colour readings at 520 nm.

The data in this experiment also showed that ageing temperature had a pronounced effect on the final colour measurements investigated. Regarding the effect of ageing temperature, wines aged at 30 °C had the lowest CD and highest PA. This agrees with other published studies that demonstrate the same trend (lower CD and higher PA) with higher ageing temperature (Dallas et al., 1994; Gómez-Plaza et al., 2000).

3.2. Evolution of phenolic compounds during wine ageing

Phenolic compounds are involved in the changes that take place during wine ageing as they influence the colour, astringency, bitterness, oxidation level and clarity of wines. The results presented (Table 2) showed that irrespective of ageing conditions, there was a gradual decrease in the concentrations of phenolic compounds apart from gallic acid. In a previous study, significant decreases in flavanols (glycosides) and anthocyanins content were observed in three different grape varieties (Carbenet Sauvignon, Tempranillo and Graciano) after 26 months of bottle ageing (Monagas, Bartolomé & Gómez-Cordovés, 2005). There was however, no significant change in the final concentrations of total hydroxybenzoic acids although a slight increase in gallic acid concentration was seen in Granciano and Carbernet Sauvignon wines. In this study, the increase in gallic acid may be the outcome of the breakdown of hydrolysable tannins as observed in wines processed from pomegranate (Mena, Gironés-Vilaplana, Martí & García-Viguera, 2012).

For hydroxycinnamic acids, Monagas et al. (2005) found an increase in caffeic acid, attributing the increase to be from the hydrolysis of caftaric acid. The increase in caffeic acid during bottle ageing of grape wines has also been reported (Ginjom, D'Arcy, Caffin & Gidley, 2011). In contrast, Gutiérrez et al. (2005) found that the concentration of caffeic acid increased within the first three months of ageing, thereafter reducing following 9 months of

ageing. In this study, caffeic acid concentration dropped significantly after ageing at the temperature conditions investigated and the reduction could be because they undergo polymerization reactions, and are involved in the formation of new stable anthocyanin-derived pigments through condensation reactions with anthocyanins (Gómez-Plaza et al., 2000). These so called "new pigments" are believed to be responsible for maintaining and stabilizing colour intensity in aged wines (González-Neves, Favre & Gil, 2014; Somers et al., 2015; Sun et al., 2005). Furthermore, the reduction in concentration of the glycosylated flavanols, myrecetin-3-arabinogalactoside and quercetin-3-sambubioside as seen in this research is most likely due to hydrolysis, which transforms these compounds to their corresponding aglycones (Alén-Ruiz, García-Falcón, Pérez-Lamela, Martínez-Carballo & Simal-Gándara, 2009).

As anticipated, the content of anthocyanins underwent the largest decrease and by the end of ageing, the concentration of monomeric anthocyanins delphinidin-3-*O* sambubioside and cyanidin 3-*O*-sambubioside had dropped by over 90 % in wines stored at 30 °C, while wines kept at 6 °C retained about half of their starting concentrations. The concentration of anthocyanins during the reaction kinetics was calculated using the standard curves of authentic standards of delphinidin-3-*O* sambubioside and cyanidin 3-*O*-sambubioside as described by Ifie et al. (2016). The degradation reactions of the anthocyanins followed first order kinetics (Figure 1C) which agrees with a previous work on grape wine (Mateus & de Freitas, 2001). The reaction rate constants (k) and half-life (the time taken for the concentration of the individual anthocyanin to reach half of its value),

calculated using the mathematical equations ln (At/Ao) =-k.t and (T1/2) = ln (2)/k, respectively showed that the degradation rate constants and half-life were higher with increasing ageing temperatures (Table 3) and this is in agreement with a previous study on

anthocyanins in *H. sabdariffa* (Gradinaru, Biliaderis, Kallithraka, Kefalas & Garcia-Viguera, 2003).

3.3. Identification of anthocyanin derivatives in roselle wine during ageing

The profile of some anthocyanin-derived pigments in roselle wine, whose structural identities (Supplementary 1&2) were putatively identified using HPLC-ESI-Q/TOFMS analysis are reported for the first time in roselle wine (Table 4). The compounds identified belong to the group of pyranoanthocyanins formed between the direct reaction between free anthocyanins and yeast by-products, such as acetaldehyde, pyruvic acid and vinylphenols. The occurrence of similar anthocyanin-derived pigments have been reported in grape wine and their stability and resistance to bleaching by sulphite has also been established (Vivar-Quintana, Santos-Buelga & Rivas-Gonzalo, 2002). The identification of the annotated compounds (Figure 1A) was based on their high resolution, accurate mass MS and MS/MS analysis along with their simultaneously acquired UV/Vis data. The UV/visible signals clearly show (Figure 1A) that most of the compounds had absorption at 420 nm and/or 520 nm, contributing to CD and PA readings.

Peaks 1 and 2 were identified as the parent anthocyanins, (delphinidin 3-*O*-sambubioside & cyanidin 3-*O*-sambubioside), while peak 3 was identified as the pyranoanthocyanin adduct (vitisin A type) resulting from the reaction of pyruvic acid with anthocyanins, (delphinidin 3-*O*-sambubioside in this study) as first mentioned by (Bakker & Timberlake, 1997). The formation of vitisin A (malvidin-3-monoglucoside pyruvic acid adduct) results from cyclisation between C-4 and the hydroxyl group at C-5 of the original flavylium moiety with the double bond of the enolic form of pyruvic acid, followed by dehydration and rearomatisation steps (Fulcrand, Benabdeljalil, Rigaud, Cheynier & Moutounet, 1998).

The TOFMS spectra of the UV/Vis peaks 4 and 5 revealed compounds with formulae $C_{28}H_{29}O_{16}^{+}$ and $C_{28}H_{29}O_{15}^{+}$, which were assigned as the cycloadducts of delphinidin 3-*O* sambubioside and cyanidin 3-*O*-sambubioside with acetaldehyde (vitisin B type). The targeted Q/TOFMS analysis of these compounds confirmed their supposed structures, by giving rise to fragment ions of $C_{17}H_{11}O_{7}^{+}$ and $C_{17}H_{11}O_{6}^{+}$ attributed to the pyranoanthocyanidin residue after cleavage of sambubioside moiety of delphinidin 3-*O*-sambubioside and cyanidin 3-*O*-sambubioside respectively. Furthermore, following the above detailed identification protocol relying on UV/Vis signals, TOFMS and Q/TOFMS spectra, it was also possible to identify other pyranoanthocyanins of pinotin type compounds (peaks 6-11) in roselle wine as ageing progressed (Table 3). These kind of pyranoanthocyanins are derived from the reaction of anthocyanins with hydroxycinnamic acids (caffeic, p-coumaric, ferulic acids) or their decarboxylation products mediated by yeast activities (vinylcatechol, 4-phenol and 4-vinylguaiacol) and these molecules tend to accumulate post alcohol fermentation (Cano-López et al., 2008).

The identification of anthocyanin derived pigments of pinotin type in roselle wine provides some explanation as to why the concentration of caffeic acid fell post-fermentation in this study. In Isabel wines, where the levels of hydroxycinnamic acid derivatives are high, the content of malvidin- and peonidin-based hydroxyphenyl-pyranoanthocyanins (derived from *p*-coumaric and caffeic acids) accounted for the pool of low molecular red wine pigments (Nixdorf & Hermosín-Gutiérrez, 2010).

Although the UV/Vis signal at 520 nm (Figure 1A) showed some well resolved chromatographic peaks, an unresolved “hump” appears in the chromatograms. This hump is most probably provided by a complex mixture of polymeric compounds (Kuhnert, Dairpoosh, Yassin, Golon & Jaiswal, 2013) and identifying them was beyond the scope of this study.

3.4. Changes in phenolic content and α-glucosidase inhibition during ageing of wine

One therapeutic approach to controlling type II diabetes is targeting the inhibition of intestinal enzymes (α-amylase and α-glucosidase) activities related with carbohydrate metabolism. Polyphenolic fractions from plants have attracted interest for their demonstrated potential in the inhibition of these carbohydrate digesting enzymes. In this study, the total phenolic content (TPC) in roselle wine dropped by 23.5, 25 and 47 % at the end of ageing of wines stored at 6 15 and 30 ºC respectively (Table 1). This pattern (decrease in total phenolic content) has been also been observed during ageing of grape wines and is thought to be attributed to the transformation of phenolic compounds into condensed forms that possess slightly different chemical properties and reactivities towards the Folin–Ciocalteu reagent (Garde-Cerdán, Marsellés-Fontanet, Arias-Gil, Ancín-Azpilicueta & Martín-Belloso, 2008). This reduction in total phenolic content did not impact on the bioactivity of roselle wine as the inhibition of α-glucosidase remained stable and was not significantly different ($p \geq 0.05$) from the initial value after 12 months of ageing at each of the temperatures investigated (Table 1). Although the concentrations of delphinidin 3-*O*-sambubioside and cyanidin 3-*O*-sambubioside which are mainly responsible for the inhibition (Ifie et al. 2016) reduced with ageing, it is possible that the new compounds (polymeric compounds) formed during ageing were able to inhibit the enzyme as effectively as the parent anthocyanins (delphinidin 3-*O*-sambubioside and cyanidin 3-*O*-sambubioside) and consequently maintained the inhibitory activity of the product.

3.5. Volatile composition of roselle wine after ageing

After the significant modifications in volatile composition during fermentation, chemical constituents generally react slowly during ageing to move to their equilibrium. The levels of

1-hexanol and 2-phenylethanol remained relatively stable, while there was a twofold increase in the concentration of isoamyl alcohol (Table 5). Although hydrolysis of isoamyl acetate was observed after ageing and may account for some increase in isoamyl alcohol content (precursor for the synthesis of isoamyl acetate), it is unlikely that this occurrence alone would have produced the magnitude of increase observed. It is common for wine after alcoholic fermentation to contain residual amounts of amino acid which could be transformed into their corresponding alcohols by the Ehrlich mechanism (Pozo-Bayónet al., 2005). Câmara et al. (2006) observed an increase in the concentrations of higher alcohols (isoamyl alcohol and 2–phenylethanol) in Madeira wine after 11 and 25 years of storage in oak barrels. On the contrary, some authors report the relative stability of these compounds during ageing as observed with 2-phenylethanol concentration in roselle wine in this study (Fang & Qian, 2006; Pérez-Prieto, López-Roca & Gómez-Plaza, 2003).

The results in this study also revealed decreases in fusel alcohol esters and ethyl esters (Table 5). In the course of alcoholic fermentation, esters are generally formed in excess concentrations above their equilibrium, which governs the extent of hydrolysis (law of mass action) during ageing (Pérez-Prieto et al., 2003). It is possible that these volatiles were synthesised in excess during fermentation and the subsequent reductions mainly due to chemical equilibrium adjustment. Moreover, the decrease in the contents of these esters during ageing has also been mentioned (Câmara et al., 2006). Another characteristic of the bouquet of aged wines is the increase in the content of esters of diprotic acids (Pérez-Prieto et al., 2003). Hence, it was not surprising that the concentrations of ethyl lactate and diethyl succinate (esters of diprotic acids) increased at the end of ageing.

The only volatile compound analysed in this study affected by ageing temperature was diethyl succinate which increased with higher ageing temperature. In agreement with a

previous study, wines aged at 23 °C had higher levels of diethyl succinate than their counterparts aged at 5 °C (Garde-Cerdán et al., 2008). Diethyl succinate arises from esterification of succinic acid (by-product of α-ketoglutaric by both yeast and bacteria metabolism) and increase in levels of this ester is generally observed as ageing progresses (Pérez-Coello, González-Viñas, García-Romero, Díaz-Maroto & Cabezudo, 2003;). Acetoin is produced from the reduction of diacetyl in the citric acid metabolism pathway and its concentration increased significantly ($p \leq 0.05$) after the ageing period. In a similar way, acetoin concentration were higher in wines after 10 months of ageing in both stainless steel containers and oak barrels (Liberatore, Pati, Nobile & Notte, 2010). This compound alongside diacetyl contribute to the buttery flavour and body of wines (Styger et al., 2011). Eugenol was the only terpernoid investigated in this study and the levels in wines decreased after ageing which might be due to esterification reactions.

Taken together, aroma compounds in roselle wine stored in bottles for 12 months showed decreases in both fusel alcohol acetates and ethyl esters of fatty acids, while increases occurred in the concentrations of esters of diprotic acids. In addition, higher ageing temperature resulted in increased synthesis of diethyl succinate.

4. Conclusion

The impact of ageing temperatures on the colour, phytochemical and bioactivity of roselle wine was investigated over 12 months. The data suggests that roselle wine colour, phenolic profile and subsequent α-glucosidase inhibition although relatively low, can be best maintained under low temperature (6 - 15 °C) ageing/storage conditions. The decrease in monomeric anthocyanins can be explained by the formation of new stable anthocyanin derivatives reported for the first time in roselle wine and which could be partially responsible

for maintaining the inhibitory activity. Regarding roselle wine volatiles, the changes that occurred were comparable to what obtains in grape wines, however, a sensory analysis is needed in order to ascertain whether these changes are translated into acceptability of the product. This study demonstrates that processing of *H. sabdariffa* into wine represents a promising alternative to expanding the functional properties of this crop. Future studies can investigate the impact of the raw material (fresh versus dried), oak ageing and micro-oxygenation on parameters relating to the quality of the wine including phenolic profile and content, volatile composition and health-promoting effects.

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References

Alarcon-Aguilar, F. J., Zamilpa, A., Perez-Garcia, M. D., Almanza-Perez, J. C., Romero-Nuñez, E., Campos-Sepulveda, E. A., Vazquez-Carrillo, L. I., & Roman-Ramos, R. (2007). Effect of *Hibiscus sabdariffa* on obesity in MSG mice. *Journal of Ethnopharmacology, 114*(1), 66-71.

Alén-Ruiz, F., García-Falcón, M. S., Pérez-Lamela, M. C., Martínez-Carballo, E., & Simal-Gándara, J. (2009). Influence of major polyphenols on antioxidant activity in Mencía and Brancellao red wines. *Food Chemistry, 113*(1), 53-60.

Bakker, J., & Timberlake, C. F. (1997). Isolation, identification, and characterization of new color-stable anthocyanins occurring in some red wines. *Journal of Agricultural and Food Chemistry, 45*(1), 35-43.

Câmara, J., Alves, M., & Marques, J. (2006). Changes in volatile composition of Madeira wines during their oxidative ageing. *Analytica Chimica Acta, 563*(1), 188-197.

Cano-López, M., Pardo-Mínguez, F., Schmauch, G., Saucier, C., Teissedre, P.-L., López-Roca, J. M. a., & Gómez-Plaza, E. (2008). Effect of micro-oxygenation on color and anthocyanin-related compounds of wines with different phenolic contents. *Journal of Agricultural and Food Chemistry, 56*(14), 5932-5941.

Castillo-Sánchez, J. X., García-Falcón, M. S., Garrido, J., Martínez-Carballo, E., Martins-Dias, L. R., & Mejuto, X. C. (2008). Phenolic compounds and colour stability of Vinhão wines: Influence of wine-making protocol and fining agents. *Food Chemistry, 106*(1), 18-26.

Da-Costa-Rocha, I., Bonnlaender, B., Sievers, H., Pischel, I., & Heinrich, M. (2014). Hibiscus sabdariffa L. - A phytochemical and pharmacological review. *Food Chemistry, 165*, 424-443.

Dallas, C., & Laureano, O. (1994). Effects of pH, sulphur dioxide, alcohol content, temperature and storage time on colour composition of a young Portuguese red table wine. *Journal of the Science of Food and Agriculture, 65*(4), 477-485.

Dallas, C., Ricardo-da-Silva, J. M., & Laureano, O. (1996). Products formed in model wine solutions involving anthocyanins, procyanidin B2, and acetaldehyde. *Journal of Agricultural and Food Chemistry, 44*(8), 2402-2407.

Fang, Y., & Qian, M. C. (2006). Quantification of selected aroma-active compounds in Pinot noir wines from different grape maturities. *Journal of Agricultural and Food Chemistry, 54*(22), 8567-8573.

Fulcrand, H., Benabdeljalil, C., Rigaud, J., Cheynier, V., & Moutounet, M. (1998). A new class of wine pigments generated by reaction between pyruvic acid and grape anthocyanins. *Phytochemistry, 47*(7), 1401-1407.

García-Falcón, M. S., Pérez-Lamela, C., Martínez-Carballo, E., & Simal-Gándara, J. (2007). Determination of phenolic compounds in wines: Influence of bottle storage of young red wines on their evolution. *Food Chemistry, 105*(1), 248-259.

Garde-Cerdán, T., Marsellés-Fontanet, A. R., Arias-Gil, M., Ancín-Azpilicueta, C., & Martín-Belloso, O. (2008). Effect of storage conditions on the volatile composition of

wines obtained from must stabilized by PEF during ageing without SO_2. *Innovative Food Science & Emerging Technologies, 9*(4), 469-476.

Ginjom, I., D'Arcy, B., Caffin, N., & Gidley, M. (2011). Phenolic compound profiles in selected Queensland red wines at all stages of the wine-making process. *Food Chemistry, 125*(3), 823-834.

Giusti, M. M., & Wrolstad, R. E. (2001). Characterization and measurement of anthocyanins by UV–Visible spectroscopy. R.E. Wrolstad (Ed.), *Current protocols in food analytical chemistry (Unit F1.2.1–F1.2.13). John Wiley & Sons, New York Inc*.

Gómez-Plaza, E., Gil-Muñoz, R., López-Roca, J., & Martínez, A. (2000). Color and phenolic compounds of a young red wine. Influence of wine-making techniques, storage temperature, and length of storage time. *Journal of Agricultural and Food Chemistry, 48*(3), 736-741.

Gómez-Plaza, E., Gil-Muñoz, R., López-Roca, J. M., Martínez-Cutillas, A., & Fernández-Fernández, J. I. (2002). Maintenance of colour composition of a red wine during storage. Influence of prefermentative practices, maceration time and storage. *LWT - Food Science and Technology, 35*(1), 46-53.

González-Neves, G., Favre, G., & Gil, G. (2014). Effect of fining on the colour and pigment composition of young red wines. *Food Chemistry, 157*, 385-392.

Gradinaru, G., Biliaderis, C., Kallithraka, S., Kefalas, P., & Garcia-Viguera, C. (2003). Thermal stability of *Hibiscus sabdariffa* L. anthocyanins in solution and in solid state: effects of copigmentation and glass transition. *Food Chemistry, 83*(3), 423-436.

Gutiérrez, I. H. n., Lorenzo, E. S.-P., & Espinosa, A. V. (2005). Phenolic composition and magnitude of copigmentation in young and shortly aged red wines made from the cultivars, Cabernet Sauvignon, Cencibel, and Syrah. *Food Chemistry, 92*(2), 269-283.

Ifie, I., Marshall, L. J., Ho, P., & Williamson, G. (2016). Hibiscus sabdariffa (Roselle) Extracts and Wine: Phytochemical Profile, Physicochemical Properties and Carbohydrase Inhibition. *Journal of Agricultural and Food Chemistry*.

Kuhnert, N., Dairpoosh, F., Yassin, G., Golon, A., & Jaiswal, R. (2013). What is under the hump? Mass spectrometry based analysis of complex mixtures in processed food–lessons from the characterisation of black tea thearubigins, coffee melanoidines and caramel. *Food & Function, 4*(8), 1130-1147.

Liberatore, M. T., Pati, S., Nobile, M. A. D., & Notte, E. L. (2010). Aroma quality improvement of Chardonnay white wine by fermentation and ageing in barrique on lees. *Food Research International, 43*(4), 996-1002.

Mateus, N., & de Freitas, V. (2001). Evolution and stability of anthocyanin-derived pigments during port wine aging. *Journal of Agricultural and Food Chemistry, 49*(11), 5217-5222.

Mena, P., Gironés-Vilaplana, A., Martí, N., & García-Viguera, C. (2012). Pomegranate varietal wines: Phytochemical composition and quality parameters. *Food Chemistry, 133*(1), 108-115.

Monagas, M., Bartolomé, B., & Gómez-Cordovés, C. (2005). Evolution of polyphenols in red wines from *Vitis vinifera* L. during aging in the bottle. *European Food Research and Technology, 220*(3-4), 331-340.

Mounigan, P., & Badrie, N. (2007). Physicochemical and sensory quality of wines from red sorrel/roselle (*Hibiscus sabdariffa* L.) calyces: effects of pretreatments of pectolase and temperature/time. *International Journal of Food Science & Technology, 42*(4), 469-475.

Nixdorf, S. L., & Hermosín-Gutiérrez, I. (2010). Brazilian red wines made from the hybrid grape cultivar Isabel: Phenolic composition and antioxidant capacity. *Analytica Chimica Acta, 659*(1–2), 208-215.

Odigie, I., Ettarh, R., & Adigun, S. (2003). Chronic administration of aqueous extract of *Hibiscus sabdariffa* attenuates hypertension and reverses cardiac hypertrophy in 2K-1C hypertensive rats. *Journal of Ethnopharmacology, 86*(2), 181-185.

Peng, C.-H., Chyau, C.-C., Chan, K.-C., Chan, T.-H., Wang, C.-J., & Huang, C.-N. (2011). *Hibiscus sabdariffa* polyphenolic extract inhibits hyperglycemia, hyperlipidemia, and glycation-oxidative stress while improving insulin resistance. *Journal of Agricultural and Food Chemistry, 59*(18), 9901-9909.

Pérez-Coello, M. S., González-Viñas, M. A., García-Romero, E., Díaz-Maroto, M. C., & Cabezudo, M. D. (2003). Influence of storage temperature on the volatile compounds of young white wines. *Food Control, 14*(5), 301-306.

Pérez-Prieto, L. J., López-Roca, J. M., & Gómez-Plaza, E. (2003). Differences in major volatile compounds of red wines according to storage length and storage conditions. *Journal of Food Composition and Analysis, 16*(6), 697-705.

Pozo-Bayón, M., G-Alegría, E., Polo, M., Tenorio, C., Martín-Álvarez, P., Calvo De La Banda, M., Ruiz-Larrea, F., & Moreno-Arribas, M. (2005). Wine volatile and amino acid composition after malolactic fermentation: effect of *Oenococcus oeni* and *Lactobacillus plantarum* starter cultures. *Journal of Agricultural and Food Chemistry, 53*(22), 8729-8735.

Rommel, A., Heatherbell, D., & Wrolstad, R. (1990). Red raspberry juice and wine: effect of processing and storage on anthocyanin pigment composition, color and appearance. *Journal of Food Science, 55*(4), 1011-1017.

Singleton, V., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*(3), 144-158.

Somers, T., & Pocock, K. (2015). Evolution of red wines III. Promotion of the maturation phase. *VITIS-Journal of Grapevine Research, 29*(2), 109.

Styger, G., Prior, B., & Bauer, F. F. (2011). Wine flavor and aroma. *Journal of Industrial Microbiology & Biotechnology, 38*(9), 1145-1159.

Sumby, K. M., Grbin, P. R., & Jiranek, V. (2010). Microbial modulation of aromatic esters in wine: Current knowledge and future prospects. *Food Chemistry, 121*(1), 1-16.

Sun, B., & Spranger, M. I. (2005). Changes in phenolic composition of Tinta Miúda red wines after 2 years of ageing in bottle: Effect of winemaking technologies. *European Food Research and Technology, 221*(3), 305-312.

Vivar-Quintana, A., Santos-Buelga, C., & Rivas-Gonzalo, J. (2002). Anthocyanin-derived pigments and colour of red wines. *Analytica Chimica Acta, 458*(1), 147-155.

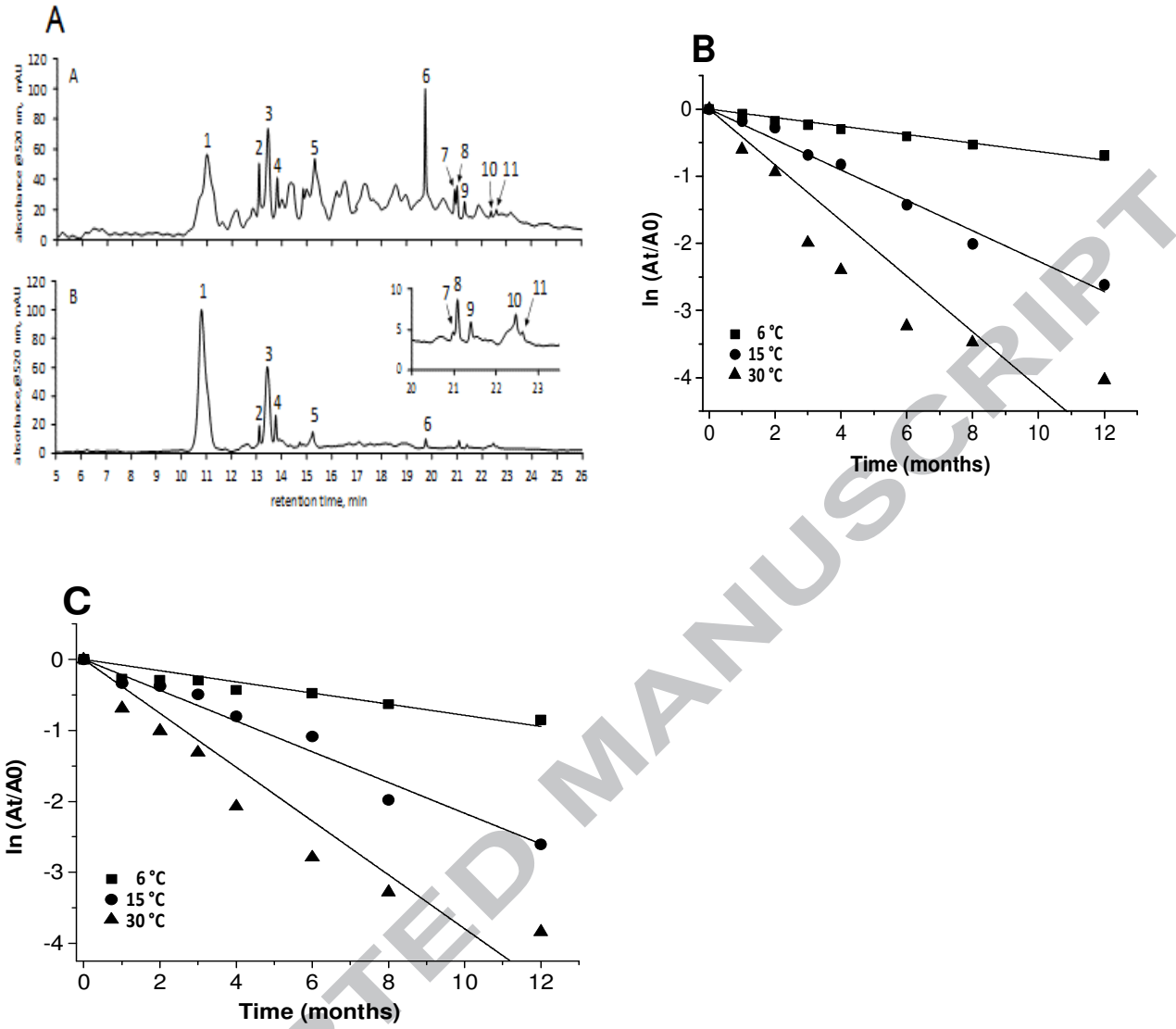

Figure 1 (A) HPLC chromatogram showing the anthocyanins and pyranoanthocyanins detected in roselle wine at 12 months. (B) & (C) show the first-order kinetic plots for anthocyanins in roselle wines.

Highlights

- Eight anthocyanin-derived compounds are reported for the first time in roselle wine
- Anthocyanin degradation during ageing is seen to follow first order kinetics
- Ageing does not diminish α-glucosidase inhibition activity of roselle wine
- Diethyl succinate is the only volatile influenced by ageing temperature

Table 1. Changes in wine colour attributes, total phenolic content and α–glucosidase inhibitionduring bottle ageing at different temperatures.

colour indices/ageing temperature (°C)	changes during ageing of roselle wine				
	after fermentation	day 60	day 120	day 240	day 360
colour density					
6	21.80 ± 0.20 $^{\alpha}$	20.16 ± 0.23	20.25 ± 0.28	17.76 ± 0.62	16.76 ± 0.03 $^{a, \beta}$
15	21.80 ± 0.20 $^{\alpha}$	19.15 ± 0.13	18.05 ± 0.25	15.30 ± 0.15	14.31 ± 0.02 $^{b, \beta}$
30	21.80 ± 0.20 $^{\alpha}$	17.47 ± 1.00	13.59 ± 0.07	10.48 ± 0.37	9.54 ± 0.58 $^{c, \beta}$
hue tint					
6	0.36 ± 0.02 $^{\alpha}$	0.35 ± 0.01	0.41 ± 0.01	0.42 ± 0.10	0.43 ± 0.01 $^{c, \beta}$
15	0.36 ± 0.02 $^{\alpha}$	0.37 ± 0.00	0.45 ± 0.01	0.51 ± 0.13	0.55 ± 0.01 $^{b, \beta}$
30	0.36 ± 0.02 $^{\alpha}$	0.42 ± 0.00	0.58 ± 0.00	0.61 ± 0.02	0.68 ± 0.02 $^{a, \beta}$
polymeric anthocyanin (%)					
6	12.70 ± 0.80 $^{\alpha}$	22.17 ± 0.39	20.78 ± 0.34	31.40 ± 0.60	32.30 ± 5.06 $^{c, \beta}$
15	12.70 ± 0.80 $^{\alpha}$	27.58 ± 0.25	31.18 ± 0.22	47.95 ± 5.60	63.40 ±1.64 $^{b, \beta}$
30	12.70 ± 0.80 $^{\alpha}$	33.18 ± 1.64	53.44 ± 0.96	67.14 ± 0.13	96.75 ± 4.02 $^{a, \beta}$
total phenolic content(mg/L)					
6	1363 ± 44.00 $^{\alpha}$	837 ± 20.00	890 ± 63.00	891 ± 28.00	1042 ± 200.00 $^{a, \beta}$
15	1363 ± 44.00 $^{\alpha}$	933 ± 33.00	840 ± 17.00	819 ± 52.00	1022 ± 15.00 $^{a, \beta}$
30	1363 ± 44.00 $^{\alpha}$	959 ± 50.00	884 ± 14.00	822 ± 62.00	960 ± 26.00 $^{a, \beta}$
α–glucosidase inhibition (%)					
6	29.69 ± 0.64 $^{\alpha}$	32.98 ±3.54	37.16 ±3.20	32.10 ±5.12	32.3±5.22 $^{a, \alpha}$
15	29.69 ± 0.64 $^{\alpha}$	37.16 ±5.38	39.70±4.80	35.82 ±1.46	28.10±1.22 $^{a, \alpha}$
30	29.69 ± 0.64 $^{\alpha}$	28.54 ±0.79	33.28 ±3.50	26.11 ±3.49	26.63±1.90 $^{a, \alpha}$

Values with different letters and Greek alphabet are significantly different between ageing temperatures and months respectively $p \leq 0.05$ (n = 6).

Table 2. Changes in phenolic compounds during ageing at different temperature.

compound/ageing temperature (°C)	content in mg/L during months of ageing							
	after fermentation	day 30	day 60	day 90	day 120	day 180	day 240	day 360
gallic acid								
6	9.60 ± 0.04 $^{\alpha}$	9.6 ± 0.1	10.14 ± 1.01	10.17 ± 0.05	10.35 ± 0.10	10.65 ± 0.63	10.35 ± 0.09	10.80 ± 0.23 $^{b, \alpha}$
15	9.60 ± 0.04 $^{\alpha}$	10.0 ± 0.1	10.28 ± 1.03	10.23 ± 0.07	10.30 ± 0.10	10.89 ± 0.85	10.34 ± 0.23	10.34 ± 0.23 $^{b, \alpha}$
30	9.60 ± 0.04 $^{\alpha}$	10.2 ± 0.3	10.88 ± 1.12	10.70 ± 0.14	11.21 ± 0.12	13.55 ± 0.34	14.30 ± 0.51	12.38 ± 0.35 $^{a, \beta}$
caffeic acid								
6	93.0 ± 4.7 $^{\alpha}$	88.32 ± 8.86	83.9 ± 1.36	83.1 ± 7.0	81.40 ± 2.72	81.04 ± 1.91	68.79 ± 1.58	60.04 ± 1.52 $^{a, \beta}$
15	93.0 ± 4.7 $^{\alpha}$	92.09 ± 5.30	80.6 ± 3.21	78.3 ± 0.1	78.21 ± 0.30	82.23 ± 2.31	68.27 ± 3.30	61.27 ± 1.11 $^{a, \beta}$
30	93.0 ± 4.7 $^{\alpha}$	91.09 ±13.06	78.05 ± 1.41	75.1 ± 1.5	73.09 ± 1.40	74.16 ± 2.42	67.68 ± 0.48	45.84 ± 0.78 $^{b, \beta}$
delphinidin 3-*O*-sambubioside								
6	318 ± 4.0 $^{\alpha}$	295.10 ± 6.11	269.0 ± 3.5	252.00 ± 5.7	235.50 ± 3.54	212.54 ± 4.19	187.32 ± 7.98	158.7 ± 20.5 $^{a, \beta}$
15	318 ± 4.0 $^{\alpha}$	265.80 ± 11.30	222.4 ± 3.2	160.8 ± 2.32	139.32 ± 9.96	76.62 ± 8.50	42.8 ± 5.03	23.24 ± 4.08 $^{b, \beta}$
30	318 ± 4.0 $^{\alpha}$	174.30 ± 5.55	87.4 ± 1.4	43.4 ± 2.05	28.89 ± 2.29	12.59 ± 0.30	9.84 ± 0.47	5.63 ± 0.20 $^{b, \beta}$
cyanidin 3-*O*-sambubioside								
6	112 ± 12.0 $^{\alpha}$	85.36 ± 1.80	83.5 ± 1.5	73.02 ± 0.54	72.85 ± 0.58	65.11 ± 1.04	59.51 ± 2.86	47.85 ± 4.92 $^{a, \beta}$
15	112 ± 12.0 $^{\alpha}$	80.09 ± 5.30	68.5 ± 1.6	50.15 ± 0.98	39.59 ± 2.16	29.60 ± 0.78	15.51 ± 2.45	8.27 ± 0.34 $^{b, \beta}$
30	112 ± 12.0 $^{\alpha}$	56.18 ± 8.16	30.2 ± 0.4	14.1 ± 0.35	10.05 ± 0.95	5.01 ± 0.13	4.21 ± 0.27	2.41 ± 0.15 $^{b, \beta}$
myrecetin-3-arabinogalactoside								
6	5.8 ± 0.4 $^{\alpha}$	5.53 ± 0.29	5.5 ± 1.26	5.35 ± 0.1	5.48 ± 0.31	5.22 ± 0.08	4.43 ± 0.37	4.32 ± 1.69 $^{a, \beta}$
15	5.8 ± 0.4 $^{\alpha}$	5.59 ± 0.43	5.53 ± 1.30	5.2 ± 0.16	5.10 ± 0.29	4.67 ± 0.04	3.70 ± 0.12	3.35 ± 1.20 $^{ab, \beta}$
30	5.8 ± 0.4 $^{\alpha}$	5.70 ± 0.49	5.01 ± 1.31	4.77 ± 0.18	4.31 ± 0.19	3.80 ± 0.08	3.37 ± 0.04	2.76 ± 0.05 $^{b, \beta}$
quercetin-3-sambubioside								
6	5.3 ± 0.1 $^{\alpha}$	4.38 ± 0.18	4.70 ± 1.76	4.57 ± 0.17	4.43 ± 0.11	4.28 ± 0.09	3.58 ± 0.04	3.87 ± 0.44 $^{a, \beta}$
15	5.3 ± 0.1 $^{\alpha}$	4.62 ± 0.61	4.80 ± 1.80	4.56 ± 0.16	4.27 ± 0.54	3.95 ± 0.66	3.19 ± 0.32	2.44 ± 0.08 $^{b, \beta}$
30	5.3 ± 0.1 $^{\alpha}$	5.60 ± 0.60	4.70 ± 1.51	4.81 ± 0.06	4.36 ± 0.58	2.90 ± 0.02	2.47 ± 0.06	2.42 ± 0.15 $^{c, \beta}$

Values with different letters and Greek alphabet are significantly different between ageing temperatures and months respectively $p \leq 0.05$ (n = 6).

Table 3. Degradation rate constants (k; month^{-1}), half-life ($T_{1/2}$; month) of roselle wine anthocyanins aged at different ageing temperatures.

	anthocyanin					
	delphinidin 3-*O*-sambubioside			cyanidin 3-*O*-sambubioside		
ageing temperature (°C)	k	R^2	$T_{1/2}$ (months)	k	R^2	$T_{1/2}$ (months)
6	0.06	0.968	11.95	0.07	0.830	9.90
15	0.22	0.985	3.15	0.22	0.973	3.15
30	0.34	0.826	2.03	0.32	0.900	2.16

peak no	retention time	measured precursor *m/z*	ion formula	exact mass	error, ppm	base peak in MS/MS	Ion formula	exact mass	error, ppm	putatively identified compound
1	10.97	597.1460	$C_{26}H_{29}O_{16}$	597.1450	1.67	303.0489	$C_{15}H_{11}O_7$	303.0499	-3.30	dp 3-*O*-sambubioside
2	13.06	665.1349	$C_{29}H_{29}O_{18}$	665.1348	0.15	371.0385	$C_{18}H_{11}O_9$	371.0398	-3.50	carboxy-pyrano- dp-samb
3	13.42	581.1506	$C_{26}H_{29}O_{15}$	581.1501	0.86	287.0553	$C_{15}H_{11}O_6$	287.0550	1.05	cyanidin 3- *O*-sambubioside
4	13.79	621.1449	$C_{28}H_{29}O_{16}$	621.1450	-0.16	327.0528	$C_{17}H_{11}O_7$	327.0499	8.87	pyrano-dp- sambubioside
5	15.29	605.1518	$C_{28}H_{29}O_{15}$	605.1501	2.81	311.0550	$C_{17}H_{11}O_6$	311.0550	0.00	pyrano-cy- sambubioside
6	19.79	729.1654	$C_{34}H_{32}O_{18}$	729.1661	-0.96	435.0694	$C_{23}H_{15}O_9$	435.0711	-3.91	pyrano-dp-catechol-sambubioside
7	20.97	713.1701	$C_{34}H_{33}O_{17}$	713.1712	-1.54	419.0753	$C_{23}H_{15}O_8$	419.0761	-1.91	pyrano-dp-phenol- sambubioside
8	21.06	713.1725	$C_{34}H_{33}O_{17}$	713.1712	1.82	419.0737	$C_{23}H_{15}O_8$	419.0761	-5.73	pyrano-cy-catechol- sambubioside
9	21.37	743.1811	$C_{35}H_{35}O_{18}$	743.1818	-0.94	449.0853	$C_{24}H_{17}O_9$	449.0867	-3.12	pyrano-dp-guaiacol- sambubioside
10	22.45	697.1760	$C_{34}H_{33}O_{16}$	697.1763	-0.43	403.0806	$C_{23}H_{15}O_7$	403.0812	-1.49	pyrano-cy-phenol- sambubioside
11	22.65	727.1872	$C_{35}H_{35}O_{17}$	727.1869	0.41	433.0916	$C_{24}H_{17}O_8$	433,0918	-0.46	pyrano-cy-guaiacol- sambubioside

Table 4. Identification of anthocyanins and pyranoanthocyanins in aged roselle wines according to their UV/vis and ESI-Q/TOFMS data.

dp; delphinidin, cy; cyanidin

Table 5. Content of volatile compounds in roselle wine after ageing for 12 months.

volatile compound (ug/L)	qualifying ions	after fermentation	ageing temperatures		
			6 °C	15 °C	30 °C
2-phenylethanol	**91**;92; 122	527 ± 63 [a]	521 ± 52 [a]	564 ± 140 [a]	483 ± 45 [a]
isoamyl alcohol	**71**; 43; 55	130539 ± 21943 [b]	269347 ± 23174 [a]	295046 ± 64401 [a]	252548 ± 7622 [a]
1-hexanol	**56**; 43; 69	248 ± 103 [a]	149 ± 34 [b]	153 ± 79 [b]	103 ± 33 [b]
ethyl acetate	**43**; 61; 70	156 ± 32 [a]	184 ± 45 [a]	226 ± 126 [a]	227 ±11 [a]
ethyl hexanoate	**88**; 99; 145	3487 ± 363[a]	1703 ± 199[b]	1718 ± 459 [b]	1634 ± 339 [b]
ethyl octanoate	**88**; 101; 172	1339 ± 135 [a]	731 ± 105 [b]	803 ± 163 [b]	707 ± 31 [b]
ethyl decanoate	**88**; 101; 200	966 ± 95 [a]	610 ± 81 [b]	607 ± 121 [b]	554 ± 36 [b]
diethyl succinate[a]	**101**;129; 55	0.003[d]	0.24 [c]	0.40 [b]	0.77 [a]
ethyl lactate[a]	**45;** 73;43	0.13 [b]	0.16 [ab]	0.21 [a]	0.19 [a]
isoamylacetate[a]	**70**;41; 88	0.69 [a]	0.15 [b]	0.18 [b]	0.15 [b]
methyl butyrate[a]	**43**;71;88	0.010 [c]	0.023 [b]	0.035 [a]	0.034 [a]
eugenol[a]	**164**; 149; 103	0.013 [a]	0.002 [b]	0.002 [b]	0.002 [b]
2-phenyl acetate[a]	**104**;91; 65	0.07 [a]	0.007 [b]	0.007 [b]	0.006 [b]
acetoin[a]	**45;** 43; 88	0.046 [b]	0.26 [a]	0.23 [a]	0.21 [a]

The values are the mean ± SD. Numbers bolded were used as the quantitative ion for aroma compounds; [a] Compounds measured by semi-quantification; relative peak areas (compound: internal standard) were used to calculate means and standard deviation .Values with different letters on the same row are significantly different $p \leq 0.05$ (n = 6).