Food Chemistry 190 (2016) 896–903

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

# New grape stems-based liqueur: Physicochemical and phytochemical evaluation

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ARTICLE INFO

*Article history:*
Received 18 January 2015
Received in revised form 15 June 2015
Accepted 16 June 2015
Available online 17 June 2015

*Keywords:*
*Vitis vinifera* L.
Wine-industry by-products
Antioxidant activity
Phenolic compounds
Liqueur

ABSTRACT

A number of traditional liqueurs are obtained by maceration of red fruits in aqueous ethanol liquor, namely sloe berries or sour cherry. On the other hand, the exploration of residual plant material derived from the winery industry (grape (*Vitis vinifera* L.) stems), which has been regarded as an interesting source of colored and uncolored (poly)phenols, could lead to an industrial alternative to the traditional distilled spirits produced, with valuable physicochemical and phytochemical properties. In the present work, vinification residues (grape stems) were used to produce a new beverage. The evaluation of the physic-chemical characteristics and phytochemical composition as well as the evolution of the determined parameters during maceration (90 and 180 days) allowed a number of interesting bioactive compounds to be identified. This new beverage is a liqueur with a high retention of phenolic compounds (*ortho*-diphenols, flavanols, flavonols, and anthocyanins), with interesting physic-chemical characteristics, that revealed significant antioxidant activity.

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## 1. Introduction

To date, a number of liquor-based beverages made, for example, by the maceration of red fruits such as sloe berries (*Prunus spinosa* L.) or sour cherries (*Prunus cerasus* L.) for the production of *Pacharán* (Northern Spain) and *Ginjinha* (Portugal), respectively, are commercially available. According to data available from the Spanish sectorial ministry, the industrial production of the former liqueur may reach up to $5 \times 10^6$ L per year by itself (MAGRAMA, 2010). These beverages usually display intense red color due to the extraction of anthocyanins from sloe and sour berries during the maceration procedure. This group of phenolic compounds and other phytochemicals, which are present in the red fruits employed, besides their color properties with technological applications, deserve to be considered for their potential health benefits as previously reported (de Pascual-Teresa, Moreno, & García-Viguera, 2010).

The use of harvested grapes for wine-making processes represents a significant amount of fruits, with the residues derived from these industries reaching more than 14.5 million tons each year in Europe alone (Chouchouli et al., 2013). The seasonality of the grape production leads to an accumulation of winery residues (skins, seeds, and stems) in autumn, with up to 30% (w/w) of the plant material reaching industry during this period. These wastes have a direct impact on the sectorial sustainability and deleterious effects on the local agro-industrial environments (Arvanitoyannis, Ladas, & Mavromatis, 2006; Bustamante et al., 2008; González-Centeno et al., 2012; Makris, Boskou, & Andrikopoulos, 2007). However, these by-products might be useful as a source of potentially functional ingredients in the elaboration of innovative liqueurs, providing an opportunity to develop valuable marketable products and thus, increasing the competitiveness of the wine industry.

To date, previous evaluations of grape stems from Portuguese varieties have shown that 'Sousão' is one of the most interesting cultivars regarding the content in (poly)phenols (Barros et al., 2014). On this point, the content of bioactive phytochemicals

*Abbreviations:* GAE, gallic acid; CE, catechin; TA, titratable acidity; TSS, total soluble solids; CIE, International Commission of Illumination; OIV, International Organisation of Vine and Wine; TPC, total phenolic content; Fl, flavonoids; OdP, *ortho*-diphenols; Stl, stilbenes; Ant, Anthocyanins.

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[1] http://www.utad.pt.

http://dx.doi.org/10.1016/j.foodchem.2015.06.047
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reported in grape stems of this variety has shown much higher concentrations of bioactive compounds that surpassed other local red varieties grown under equal agro-climatic conditions. The mounts of proanthocyanins exceeded other cultivars by 16.0% on average, and the content in flavonols, *ortho*-diphenols, and anthocyanins by 49.6%, on average (Barros et al., 2014). These results show that the 'Sousão' variety is most suitable for use as an ingredient in the development of innovative and competitive commodities, including liqueurs.

With the perspective of innovation on new products with potential application in food industries, helping to reduce the disposal of the high amounts of residues derived from the vinification process, a grape stem-based liqueur was designed and evaluated on their physico-chemical characteristics, phytochemical composition (total phenolic compounds, total *ortho*-diphenols, total flavonoids, and anthocyanins), antioxidant capacity (ABTS), and phenolic profile (HPLC), as well as the evolution of the assessed parameters during maceration (90 and 180 days).

## 2. Material and methods

### 2.1. Chemical

The reagents Folin–Ciocalteau, Trolox (6-hydroxy-2,5,7,8-tetra methychroman-2-carboxylic acid), (2,2-azino-bis(3-ethyl-benzo thiazoline)-6 sulfonic acid) diammonium salt ($ABTS^{\cdot+}$), methanol, gallic acid (GAE), and catechin (CE) were all of extra pure grade (>99%), purchased from Sigma–Aldrich (St. Louis, MO, USA). Acetic acid (>99%) was purchased from Panreac (Panreac Química S.L.U., Barcelona, Spain). Sodium nitrate, aluminum chloride, and sodium carbonate, all extra pure (>99%), were purchased from Merck (Merck Darmstadt, Germany). Sodium molybdate (99.5%) was purchased from Chem-Lab (Chem-Lab N.V., Zedelgem, Belgium). All other reagents used were of analytical grade. Ultrapure water was obtained using a Millipore water purification system.

### 2.2. Beverage preparation and sample extraction

For liqueurs' processing, fresh grape (*Vitis vinifera* L.) stems from the red variety 'Sousão' (50 g) were chopped and added to 250 mL of grape marc spirits, commonly known as '*bagaceira*'. Grape marc spirits used for the elaboration of liqueurs was provided by Quinta da Nossa Senhora da Luz, Symington Family Estates, located in northern Vila Real, Portugal, being the same foodstuff employed in the elaboration of local liqueurs such as '*Ginginha*', and meeting all market safety requirements. Moreover, 100 g of sugar (regular commercially available sucrose) were added to the mixture. Then, samples were stored at 20 °C, in darkness, without $O_2$ contact, preparing three repetitions of each specific condition of maceration. Maceration containers were almost filled, providing a maximal reduction of the headspace and ensuring that the oxygen content was consumed during the 2 first week in agreement with Vidal et al. (2004) and hermetically sealed until the sampling time-point (Days 90 and 180). The liqueurs were stored for six months and analyzed at days 0, 90, and 180. Furthermore, a control sample was produced by adding solely the 100 g of sugar to 250 mL of wine spirits, without grape stems, and undertaking the same procedure as for the liqueurs. Phenolic compounds from grape stems were extracted into the liqueurs by natural leaching. Samples (1 mL) were filtered through 0.22 μm PVDF filter/Millex HV13, Millipore, Bedford, MA). Each sample (20 μL) was injected into HPLC-PDA-ESi-$MS^n$ for identification of individual phenolic compounds.

### 2.3. pH, titratable acidity (TA), total soluble solids (TSS), alcoholic, and methanolic content

The pH values were measured using a pH-meter (Jenway 3305, Felsted, UK). The TA was determined by titrating 1.0 mL of the liqueur (rising 5.0 mL final volume with Milli-Q water) with NaOH 0.1 M, until a pH of 8.2 was reached. Results were expressed as grams of tartaric acid per 100 mL of liqueur. TSS contents were recorded in a refractometer (ATAGO, Tokyo, Japan) at 20 °C with values being expressed as °Brix as previously reported (González-Molina, Moreno, & García-Viguera, 2008). Measurements were repeated twice for each replicate ($n = 3$). The alcoholic volume (%vol.) was determined according to the ebulliometric method recommended by OIV (International Organization of Vine and Wine) (Zoecklein, Fugelsang, Gump, & Nury, 1995).

The methanolic content has been assessed through FTIR-ATR coupled to multivariate analysis. For this purpose an external calibration was undertaken using fifteen distinct aqueous alcoholic solutions within a range of different methanol/ethanol ratios (0.00–0.05). Partial Least Squares regression (PLS-R) has been used to produce a model, which was validated through the cross-validation procedure, making use of the leave-one-out (LOO) approach. The frequency interval 2814–2950 $cm^{-1}$, where the symmetric/anti-symmetric ($CH_3$) stretching modes of both ethanol and methanol are present at different frequencies, was selected after preliminary tests. The first derivatives of the spectra have been used, and each spectrum has been normalized by its mean absorption. The relationship between the actual methanol contents, and the predicted values showed an $r^2$ of 0.999 for the training set, while the validation procedure retrieved a RMSECV(%) of 4.8. The multivariate detection limit was estimated to be around 0.53 g of methanol per hectoliter of ethanol.

### 2.4. Color measurements

The color of the liqueurs was evaluated by determining CIE $L^*$, $a^*$, and $b^*$. Color parameters were recorded and processed using the colorimetric system Minolta Chroma Meter CR-300 (Osaka, Japan). CIE $L^*$, $a^*$, and $b^*$ coordinates were obtained using illuminant D65 at 10° observer as reference system. The equipment was calibrated with a white standard, and $L^*$, $a^*$, and $b^*$ parameters were calculated from the average of four color measurements. From these values, *Hue angle* ($H$) was calculated from '$H = \tan^{-1}(b^{*2}/a^{*2})$' and *Chroma* ($C^*$) from '$C^* = (a^{*2} + b^{*2})^{1/2}$'.

### 2.5. Phenolic composition

The total phenolic content was determined by colorimetric analysis using Folin–Ciocalteau reagent (Singleton and Rossi, 1965). In a test tube, 6.5 mL of distilled water, 1.0 mL of sample diluted 1:50 (v/v, in MeOH/HCl (99:1, v/v)), and 500 μL of Folin–Ciocalteau reagent were added. After 3 min, 2.0 mL of 7.5% (w/v) aqueous sodium carbonate were added into each tube, which was agitated in a vortex, and afterwards incubated at 70 °C for 30 min. After cooling down samples at room temperature, the absorbance was measured on spectrophotometer at 750 nm (all absorbance measurements were taken using a Spectronic Genesys™ 20 Visible Spectrophotometer 4001-000, France).

The total *ortho*-diphenol content was assessed by adding 1.0 mL of a 5.0% solution of $Na_2MoO_4$ (w/v) in 49 mL of MeOH/$H_2O$ (50:50, v/v), to 4.0 mL of sample diluted 1:99 (v/v, in MeOH/HCl (99:1, v/v)). After 15 min, the absorbance was measured at 370 nm (Mateos et al., 2001).

The content in total phenolics and *ortho*-diphenols were expressed as milligrams of gallic acid equivalents per 100 mL of liqueur. Concentrations were calculated on freshly prepared

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