Food Research International 100 (2017) 469–476

Contents lists available at ScienceDirect

Food Research International

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

Metabolic fingerprinting of must obtained from sun-dried grapes of two indigenous Cypriot cultivars destined for the production of 'Commandaria': A protected destination of origin product

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ARTICLE INFO

Article history:
Received 20 April 2016
Received in revised form 20 October 2016
Accepted 15 November 2016
Available online 24 November 2016

Keywords:
Vitis vinifera
Autochthonous cultivars
Phenolic compounds
Sun-drying
Dessert wine
LC-DAD-qTOF-MS

ABSTRACT

Grapes's sun-dried process is one of the most critical steps in the production of 'Commandaria', a dessert wine product that is exclusively produced in Cyprus and derived from must obtained from two indigenous grape cultivars, namely 'Mavro' and 'Xynisteri'. Despite its significant economic importance, no data regarding the primary and secondary metabolites of the aforementioned cultivars exist. Thus, in the current study, the effect of sun drying process on the qualitative attributes and phenolic profile of 'Mavro' and 'Xynisteri' musts was dissected. Musts were analyzed at harvest and at the end of the sun-drying process that corresponds to ca. 30–40% water loss. Results highlighted significant differences in chemical composition of the must before and after the sun-drying process. Except for the increase of soluble solids content, a significant increment in glucose, fructose, total acidity, total phenols and total flavonoids contents was monitored. Subsequently, forty-two phenolic compounds were identified by LC-DAD-qTOF-MS revealing the polyphenolic fingerprint of the two cultivars. Results also indicated that changes in the phenolic composition of the obtained must are not only correlated with the dehydration effect, but both synthesis or degradation reactions occurred. In particular, the increases in the concentration of hydroxybenzoic acids were higher than the concentration effect for both cultivars. Regarding to hydroxycinnamates, dehydration caused a six-fold increase of hydroxycinnamic acid content in both cultivars. Intriguingly, the concentration of some hydroxycinnamic acids such as caffeic acid dihexoside and fertaric acid isomer went descending. Although the degradation of the internal side of the skin facilitate improved extractability from the skins to the grape pulp and therefore to the grape must, the sun-drying process may also induced stilbene and lignans synthesis production. A significant effect of dehydration on the postharvest biosynthesis of three groups of flavonoids (flavonols, flavan-3-ols, flavanonols), was also observed. This study sheds some light in the substantial changes that occur in specific metabolites during the sun drying process; such metabolites can be considered as potential factors that may determine organoleptic characteristics and biological properties of the end-product.

1. Introduction

'Commandaria' is an amber-coloured dessert wine produced in Cyprus since 4000 years ago, being considered a protected designation of origin (PDO) product. According to Cyprus legislation, its production is only allowed in a specific area of fourteen villages, located on the foothills of the Troodos Mountain at an altitude from 500 to 900 m. 'Commandaria' is exclusively produced through the fermenting process of must obtained from sun-dried grapes of two indigenous grape cultivars, namely 'Xynisteri' (white grapes) and 'Mavro' (red grapes) (Ioannou-Papayianni, Kokkinofta, & Theocharis, 2011).

The production process of 'Commandaria' starts with the sun-drying of the grapes for usually 10 to 15 days, depending on the climatic conditions. The optimum conditions for sun-drying require high diurnal temperatures and low ambient moisture. Grapes are spread to nets placed in large open sites, specifically suitable for this purpose on account of their orientation and gentle slope. During the drying process, the grapes are turned over by hand periodically in order to have a uniform concentration of their components. A critical point during this process is that berry drying has to be carried out moderately in order to avoid excessive evaporation which may adversely affect the pressing process.

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http://dx.doi.org/10.1016/j.foodres.2016.11.015
0963-9969/

In general, a direct exposure to sunlight results to a significant water loss in the grape berry and several changes in its chemical composition. In particular, the sun-drying process causes grape skin to gradually shrink, darken and become less elastic and more fragile; accordingly, berry pulp is also shrunken and darkened (De Lerma, Moreno, & Peinado, 2014). In addition, grape dehydration causes substantial alterations in its metabolism, due to fruit exposure at abiotic stress conditions (Marquez, Dueñ, Serratosa, & Merida, 2012a). Previous study reported that the stress situation occur when the weight loss is 10–15%, causing a change from aerobic to anaerobic metabolism (Bellincontro, De Santis, Botondi, Villa, & Mencarelli, 2004). The secondary metabolites, such as polyphenols and volatiles, are also influenced by the dehydration process of grapes (Chkaiban et al., 2007). As a general rule, the off-vine dehydration produces very dark must, richer in sugars and aromatic substances (Ruiz, Zea, Moyano, & Medina, 2010). Regarding acidity, a slight increase of the concentration of organic acids usually occurs as a result of water loss. However, when the dehydration process is slow, a reduction of organic acids occurs, being attributed to anaerobic metabolism of the grapes that subsequently leads to malic acid degradation (Panceri, Gomes, De Gois, Borges, & Bordignon-Luiz, 2013). A significant alteration of color parameters in dried grapes is also monitored, as a result of both, enzymatic action (i.e. polyphenol oxidase) and non-enzymatic browning reactions (i.e. Maillard reaction), yielding coloured products known as melanoidins (Marquez et al., 2012a). Finally, a great impact of dehydration on grape polyphenols has been found. Except for the concentration of phenolic compounds during drying, their enzymatic degradation and postharvest biosynthesis has also been reported (Figueiredo-Gonzalez, Cancho-Grande, & Simal-Gandara, 2013). In addition, dehydration causes a degradation of the internal side of the grape skin resulting some migration of phenolic compounds from the skin to the grape flesh. However, and despite its economic impact in Cyprus viticulture, no details are available about the polyphenolic profile of 'Mavro' and 'Xynisteri' cultivars and how this is being affected by the sun-drying process.

Thus, the objective of this work was to monitor the main compositional changes during the sun-drying process of grapes destined for the production of 'Commandaria' wine. In particular, it was studied the effect of dehydration on metabolites, such as sugars, organic acids and phenolic compounds that are mainly responsible for its special taste. In addition, this is the first attempt to characterize the must obtained from the two indigenous grape cultivars ('Xynisteri' and 'Mavro') that accounts for ca. 70% of vineyards in Cyprus.

2. Materials and methods

2.1. Fruit material and sampling procedure

'Xynisteri' and 'Mavro' grapes, free from any visual defects, were harvested at commercially maturity stage from five selected vineyards, located at 'Commandaria' protected area. Such grapes were mixed and six representative 20-kg batches per cultivar were prepared.

Freshly harvested grapes were crushed manually and pressed in a laboratory vertical press (Torchiertto Premitutto, Italy), similar to industrial models. The obtained must was clarified by centrifugation at 4700 rpm min^{-1} for 15 min and preserved at −20 °C until needed. Half of them were used to receive must after harvest and the rest after sun-drying process.

2.2. Sun-drying process

Grapes after sampling procedure were spread to nets placed in large open sites, specifically suitable for this purpose on account of their orientation and gentle slope. During the drying process, the grapes are turned over by hand periodically in order to have a uniform concentration of their components. The sun-drying process for 'Mavro' grapes and 'Xynisteri' grapes lasted 10 days and 12 days, respectively. The sun-drying process was interrupted when the soluble solids content reached ca. 36°Brix, in accordance with the relevant legislation for the production of Commandaria wine (34.0–38.5°Brix). Subsequently, sun-dried grapes were used to receive must as previously described.

2.3. Determination of qualitative attributes

Soluble solids content (SSC), reducing sugars, titratable acidity and pH were determined according to the methods described by International Organization of Vine and Wine (OIV, 2012). All measurements were carried out in triplicate. SSC was measured with a portable digital refractometer (Master Baume 2594, Atago, Japan). Glucose and fructose were analyzed by high-performance liquid chromatography (HPLC, Shimadzu Corporation, Kyoto, Japan) with a refractometric detector (RID, Shimadzu Corporation, Kyoto, Japan). Once the samples were filtered, they passed over a filter cartridge C_{18} to remove phenolic compounds. Then, a volume of 20 μL for each sample was injected into a Luna® (30 × 4.6 mm id, 5-μm) column (Phenomenex, Cheshire, UK). The elution was carried out with a mobile phase of acetonitrile/water (80/20, v/v), delivered at 1 mL min^{-1}. Titratable acidity was determined by potentiometric titration with 0.1 mol L^{-1} NaOH up to pH 8.1, using 5 mL juice diluted in distilled water until final volume of 25 mL. The measurements were carried out using a DL22 Mettler Toledo titrator (Mettler-Toledo, Inc., Columbus, Ohio, USA). The pH values were determined using a HI 2222 Hanna pH meter (Hanna instruments, Inc., Woonsocket, Rhode Island, USA)

2.4. Determination of total phenolic and total flavonoids contents

Total phenolic content (TPC) was determined using Folin-Ciocalteu's method as adopted from the International Organization of Vine and Wine (OIV, 2012) with slight modifications. Briefly, a volume of must (100 μL) was mixed with 5 mL of distilled water, 0.5 mL of Folin-Ciocalteu reagent and 2 mL of 20% *w/v* sodium carbonate. After 30 min, the mixture was monitored at 750 nm and the phenolic content was calculated as mg gallic acid equivalents (GAE) L^{-1} of must.

Total flavonoids were determined according to Hosu, Cristea, and Cimpoiu (2013). A volume of must (0.5 mL) was mixed with 0.4 mL of aluminum chloride solution (25 g/L), 0.5 mL sodium acetate (100 g/L) and 4 mL distilled water. After 15 min, the absorbance of the mixture was measured at 430 nm and the flavonoid content was expressed as mg rutin equivalents (RE) L^{-1} of must.

2.5. Identification and quantification of individual polyphenols by LC-DAD-qTOF-MS

An Agilent 1200-LC system (Agilent Technologies, Palo Alto, California, USA) equipped with a vacuum degasser, auto sampler, a binary pump, and a DAD was used for the chromatographic determination. The separation was conducted using a Poroshell 120 EC-C_{18} analytical column (4.6 mm × 100 mm, particle size 2.7 μm), operating at 25 °C and at a flow rate of 0.8 mL min^{-1}. The mobile phases used were water with acetic acid (1%, v/v) (Phase A) and acetonitrile (Phase B) and the gradient used the following conditions: 0 min, 0.8% B; 2.5 min, 0.8% B; 5.5 min, 10% B; 11 min, 10% B; 17 min, 20%B; 22 min, 30% B; 26 min, 100% B; 28 min, 100% B; 30 min, 0.8% B; and finally a conditioning cycle of 3 min with the initial conditions. A volume of 6 μL of each sample was injected. Three replicates of each extract were performed.

MS analyses were done using a 6540 Agilent Ultra-High-Definition Accurate-Mass qTOF-MS coupled to the HPLC, equipped with Agilent Dual Jet Stream electrospray ionization (Dual AJS ESI) interface. The negative ionization mode was used and the conditions were as follows: drying gas flow (N_2), 12.0 L min^{-1}; nebulizer pressure, 50 psi; gas drying temperature, 360 °C; capillary voltage, 3500 V; fragmentor voltage and scan range were 3500 V and *m/z* 50–1300, respectively. Automatic