EVALUATION OF PHENOLIC CONTENT AND COLOR PARAMETRES OF WINE PRODUCED FROM DRIED *KALLMET* GRAPE

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Abstract

Kallmet is one of the most known grape varieties in Northeast part of Albania. This area has a geographical middle-field landscape surrounded by hills, foothills and mountains. The proximity to the Adriatic Sea and Skadar Lake defines its climate, as a Mediterranean climate with mild and wet winters and with very hot and dry summers. In Mediterrean countries, drying the grapes is a known technique for producing sweet and dry wines. Different drying techniques can be utilized such as exposure to sunlight on-vine and off-vine (exp. Passito), controlled dehydration in closed chambers with warm air (exp. Amarone, Vin Santo, Recioto), or the Icewine that consist leaving the grapes to dehydrate in the vineyard, under freezing conditions. These wines are known for their different character in chemical compounds like polyphenols and their organoleptic characteristics. As Albania has favorite climate conditions for natural and not expensive drying techniques, it will be interesting introducing in Albania this new typology of wine. Polyphenols like anthocyanins, stillbenes are very important as they are known for their antioxidant activity and benefits in human health mostly in protection action against oxidative damage of lipoproteins, anti-inflammatory effect and cancer chemopreventive activity. An evaluation in these components was conducted in wines produced from dried grapes of *Kallmet*, vintage 2011, 2013. They were evaluated for their Total Tannin content, Antioxidant Activity, five most important monoglucosidated anthocyanin, Total Anthocyanin content and Color parameters. The data obtained were compared with data of wines from non dried *Kallmet* grapes which were used as white samples. It has been found that polyphenolic content of the samples were influenced by the drying process, in favor of their content. The antioxidant activity of wines does not undergoes in any significant changes with drying process and wines produced by drying the raw materials were characterized by a lower lightness.

Keywords**:** *grape, drying, wine, polyphenols, color*.

Introduction

Drying of grapes is a known technique for production of a typology of wine like sweet wines, reinforced wines and dry wines *(Scienza, 2013)*. These techniques were firstly developed in countries where the natural drying was possible to be applied. After that the techniques were improved and through the ventilated rooms, with hygroscopic controlled conditions it becomes possible production of these wines everywhere. The grape dehydration techniques currently employed can be grouped in three different types: on-vine drying, natural drying, and forced drying *(FREGONI M., 2005)*. On-vine drying has the purpose of delaying the harvest of wine grapes beyond the regular stage of technological ripening. Natural drying describes all the procedures of wine grape dehydration that are based on the favorable actions of the environmental conditions like direct grape exposure to the sun or to wind, together with the use of house garrets and house floors arranged for grape drying. Forced drying is usually used to describe the grape drying procedures that take place in closed environments which frequently have technology installed for the partial or total control of air ventilation, relative humidity and temperature. In the beginning, the drying process has been mainly considered as a process for increasing the sugar content of grape berries with little or no attention paid to the variation in other compounds induced by the modification of the cell metabolism accompanying the dehydration process. The later studies indicate that as in other pulpy fruit, grape berry development is characterized by marked changes in biochemical-physical properties, which are particularly evident during ripening *(Conde et al., 2007)*. Fruit ripening involving a series of physiological, biochemical, structural and compositional changes, all leading to the development of an edible fruit with certain desirable qualities *(Brady, 1987; Giovannoni, 2004)*. The type of drying indicates the water loss which is an element that greatly affects the general metabolism and the physiology of the berries. This is due to the lack of water, mineral and energy supply provided through the vascular connections *(Mencarelli, Bellincontro, Tonutti, & others, 2013)*. Drying temperature plays an important role in phenolic content as it can affect the secondary metabolites synthesis and the rate of oxidation of these components. Polyphenols are very important components in winemaking world. This is due to their main role in the quality of wine especially in taste, color, body and astringency *(He et al., 2012)*. In recent years it has been given a great place to study these components because of their protective role of the plant from damages as well as their role in human health. Different studies have reported evidence that phenolic compounds may exhibit a beneficial effect against cancer, protection action against oxidative damage of lipoproteins *(Doré, 2005)(Halpern, 2008)*, anti-inflammatory effect, vasorelaxation, and allergy *(Singh, Holvoet, & Mercenier, 2011; Yang, Landau, Huang, & Newmark, 2001)*. According to the role of drying after harvest in polyphenolic content in grapes and after in wine there are contradictory views in the literature, this probably due to the use of different genotypes, fruit tissue, dehydration conditions (in particular temperature) and stress intensity *(Tonutti & Bonghi, 2013)*. *Kallmet* is one of the most important autochthonous varieties in Northeast part of Albania *(Susaj, Susaj, Ferraj, & Kallço, 2012)*. The aim of this study was to test the effect of postharvest grape drying method on specific parameters of wines produced from those dried grapes. It is hypothesized that knowledge from this research could serve as the basis for providing new insights into the production of alternative wine products in Albania.

Materials and methods

Wines samples

Harvest of the *Kallmet* variety grapes was conducted in the municipalities of Hajmel, Shkodër in 2011 and 2013, respectively. After harvest, a portion (half of the total harvest), was used directly (non-dried) for vinification and this batch was used as white sample. The remaining portion was placed in an area of 25 m^2 and was left to dry in ambient conditions for 10 days in a shaded and ventilated area with temperatures of 24-26°C, until the sugar content reached from 22°Brix to 27°Brix for vintage 2011 and from 21.5°Brix to 25°Brix for vintage 2013. Then they were fermented in stainless steel tanks and after bottling stored in a cellar. Before bottling the SO_2 were adjusted to 30 mg/l.

Polyphenols

Total tannins were estimated spectrophotometrically according to a method by Porter *(Porter, Hrstich, & Chan, 1985)*, which is based on the conversion of proanthocyanidins into colored anthocyanidins by oxidative cleavage under hot and acidic conditions (100°C for 30 min in a mixture of chlorhydric acid 32%, n-butanol and ferric sulfate $Fe_2(SO_4)_3$). After the reaction the absorbance was measured at 550nm. A sample prepared with all reagents but not heated just stored at room temperature and in the darkness was used as a control. The total anthocyanin content was estimated according to Puissant-Leon by the reaction with HCl 1N *(Puissant & Léon, 1967)*. Antioxidant activity of samples was determined by DPPH and ABTS Test as described by Ginjom and expressed as EC_{50} value that is otherwise known as "effective concentration" *(Ginjom, D'Arcy, Caffin, & Gidley, 2010)*

Color Parameters

Chromatic characteristics were determined by the spectrophotometer CM-5 (KONICA-MINOLTA, Japan), and data acquisition and analysis was performed by SpectroMagic NX, Color Data Software CM-S100W (KONICA-MINOLTA, Japan). The Tristimulus reflectance colorimeter (model D25) calibrator was used. Color was recorded using the CIE-L* a* b* uniform color space (CIE-Lab), where L* indicates lightness which varies from 0 (black, bottom) to 100 (white, top), a* indicates hue on a green (-) to red (+) axis, and b* indicates hue on a blue (-) to yellow (+) axis. Chroma or C*, is the distance from the colored point to the origin of the axes and h* or hue angle, is the polar angle made by the vector of the colored point with a*+ axis. Three components yellow (A420 nm), red (A520nm), blue (A420 nm) and the color intensity (CI), Tint (T) were estimated using the method described by Ribereau-Gayon *(Ribéreau-Gayon, Dubourdieu, Doneche, & Lonvaud, 1998)*.

HPLC Analysis

Determination of three most important groups of anthocyanins in wine was performed according to the method OIV-MA-AS315-11(International Organization of Vine and Wine, Paris, 2003), which consists of Reverse Phase Chromatography (RP-HPLC), performed with the Hewlett Packard Agilent 1200 equipped with a UV-VIS detector. Data acquisition and analysis were performed by 7.1.2 Chromeleon Chromatography Data System (Dionex Softron GmbH, Germany). Separation of the components was accomplished with a silicon-based column (Zorbax Eclipse Plus C18, 50 mm, 4.6 mm, 1.8 μm). The mobile phases were: solvent A, which consisted of de-ionized water/formic acid/acetonitrile 87:10:3, and solvent B which consisted of de-ionized water /formic acid/acetonitrile 40:10:50. The solvent gradients used are shown in Table 1. The total duration of the analysis was 45 min. The volume injected was 10 µl. The flow was 2 ml/min and the column temperature 25°C. Anthocyanins were determined at 520 nm.

Results and discussion

Wine is a complex matrix, composed from a wide range of components, products of several bio-chemical reactions. There are some parameters to be measured to evaluate the quality of the wine. The most important for an immediate evaluation are pH, total acidity and alcohol content. As it can be seen in Tab.1 the pH ranged from 3.5 to 3.9. pH plays an important role in many aspects of winemaking and wine stability mostly by influencing microbiological stability, affects the equilibrium of the tartrate salts, determines the effectiveness of sulfur dioxide and enzyme additions. When comparing the pH value of Kallmet non dried (K_ND) with Kallmet dried (K_D), for both years there is no trend in pH posture. For 2011 vintage the pH was much higher in wine from dried grape and in 2013 vintage in wines from non dried grape. TA (total acidity) ranged from 5.12- 7.45 g/L and it has the same posture as pH, not showing significant changes with drying process. Alcohol, as component directly connected with sugar content in grape, it changes with the drying. As it can be seen from Tab.1 for both vintages the alcohol content in wine from dried gapes is much higher. In the first year the difference was much higher as the concentration of the sugar with the drying process was much higher.

Table 1. Chemical physical wine parameters

	pH	Total Acidity g/L	Alcohol %
K_ND _2011 n=2	3.67± 0.06	5.53±0.5	13.6±0.69
K_D_2011 n=2	3.92±0.09	5.12±0.7	17±0.14
K_ND _2013 n=2	3.8±0.43	6.15±1.07	12.1±0.28
K_D_2013 n=2	3.56±0.11	7.45±0.35	13.5±0.78

Polyphenol pattern also it is an important indicator for the value of wine. Today all the winemaking protocols tend to improve in way to increase the polyphenol content in final product. The total tannin content in Kallmet wine (Tab. 2) showed a slight decrease with drying, for vintage 2011 and an increase for vintage 2013. The total anthocyanin content pattern shows the same behavior for both vintages. With drying the anthocyanin content showed a slight decrease. It is unclear the role of drying of grapes, in phenolic content in wines. Different studies states that the amounts of anthocyanin content in the skins and in the wines are affected by the drying temperature and the weight loss of the berries. The more severe the drying process, more the oxidation reaction will take place, the synthesis reaction are slimmed down, so the content of anthocyanins can decrease (*Moreno et al., 2008*)(*Mencarelli et al., 2010*).

Table 2 Polyphenol content in Kallmet wine

	Total Tannins g/ L	Total Anthocyanins mg/L	Antioxidant activity	
			DPPH	ABTS
K_ND _2011 n=2	5.6 ± 0.57	143 ± 3.5	1.25±0.02	52.3 ± 0.57
K_D_2011 n=2	4.9 ± 0.64	141.5± 4.9	1.32 ± 0.04	41.9 ± 2.55
K_ND _2013 n=2	4.1± 0.28	345± 5.7	1.1 ± 0.03	25.6 ± 0.67
K_D_2013 n=2	5.3 ± 0.04	340±3.9	1.15 ± 0.07	14.3 ± 12

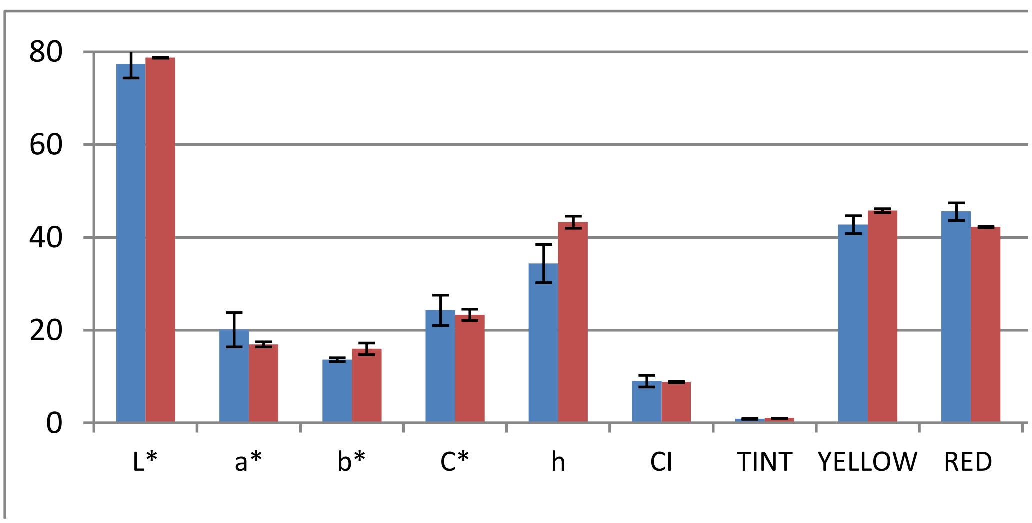
Antioxidant activity shows that, was not dependent from the drying process as it showed not significant changes between samples from dried and non dried grape. Data from literatures shows that the antioxidant activity of the wine was affected in terms of decrease only when the temperature of treatment were above 100ºC, under this temperature the changes in antioxidant activity were not significant *(Larrauri, Rupérez, & Saura-Calixto, 1997)*.

Monomeric anthocyanins are extracted from red grape berries and are the major contributors of the young red wine color. The intramolecular or intermolecular interaction of anthocyanins themselves or with other organic chemicals, especially the phenolic compounds, such as sell-association and copigmentation, can further enhance their color expression.

Table 3 Content of five monoglucosides anthocyanins

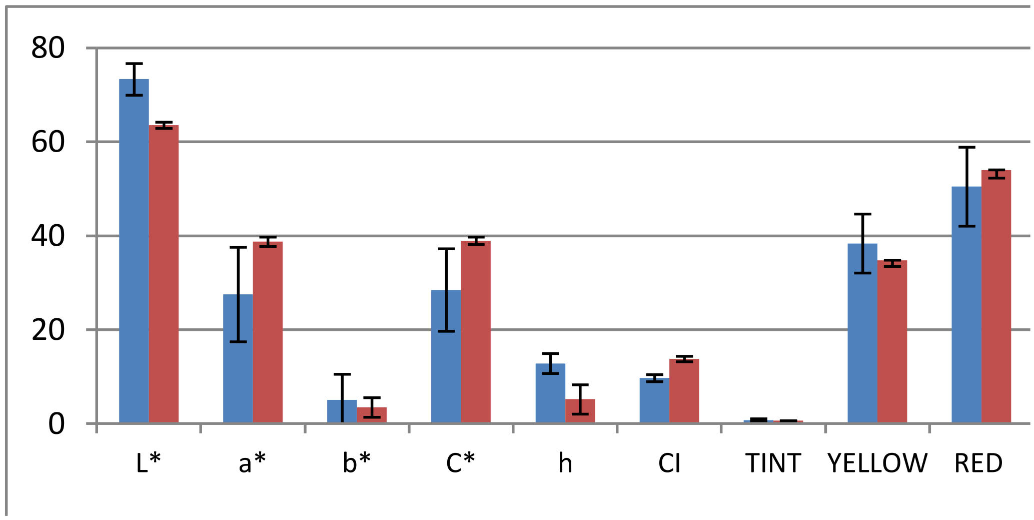
	Delphinidol -3- Glucoside	Cyanidol -3- glucoside	Petunidol -3- glucoside	Peonidol -3- glucoside	Malvidol -3- Glucoside
K_ND _2011 n=2	14.0%	0%	0.0%	11.1%	75%
K_D_2011 n=2	13.8%	0%	0.0%	10.9%	75%
K_ND _2013 n=2	2.4%	0%	6.7%	3.2%	88%
K_D_2013 n=2	4.2%	0%	8.3%	5.1%	82%

The most abundant monomeric anthocyanin in wine is **malvidol-3-glucoside** that in our samples varied from 75 % - 88% (Tab.3). For wines of 2011 vintage there were no changes in malvidin-3-glucoside content, between samples from dried and non dried grapes. And for 2013 vintage there is a slight decrease of this anthocyanin with the drying process. Delphinidol showed a slight decrease with drying for 2011 vintage from 14 % to 13.8 %, and a slight increase in 2013 vintage from 2.4 % to 4.2 %. The same pattern showed the peonidol. Petunidol showed no changes in 2011 vintage and an increase for 2013 vintage from 6.7 % to 8.3%. Differences of monomeric anthocyanins on different vintages are related with the aging process, during which the concentration of monomeric anthocyanins changes constantly, because numerous more complex and stable anthocyanin derived pigments are formed (*He et al., 2012*). Other studies showed that the temperature of drying is a significant factor for the anthocyanin content. So the Mencarelli (*Mencarelli et al., 2010*) studies, which were focused on the effect of different temperatures of drying on Aleatico winegrape phenolic fraction, concluded that the low temperature of drying but for a long time can increase the anthocyanin content. And higher temperature of drying can cause the decrease of the anthocyanin content. This is dedicated to a decrease of synthesis and an increase of degradation of these metabolites.



Graph 1 Comparison of color parameters of Kallmet 2011 wine

The color parameters shows that 2011 (Graph 1) dried wines were characterized by higher lightness (L*), a higher hue (h) and a higher content of the yellow component. This means that the wines had lighter color than wines from non dried grapes. But as it can be seen by the graph 1 the differences for lightness and yellow component it is not significant.



Graph 2 Comparison of color parameters of Kallmet 2013 wine

For the 2013, wines from dried grapes (Graph 2) were characterized by a lower lightness, which means that these wines were darker than wines from not dried grapes. Also they were more intense in color and the red component was much more abundant. So we can say that it is difficult to give trends which relate drying with the color parameters in wine. Even literature doesn't give was enabled to relate the drying effect in color parameters. The most affected was the lightness which corresponds with the data of the second year. Drying can give darker wines (*Larrauri et al., 1997*).

Conclusions

It has been found that polyphenolic content of the samples were influenced by the drying process, in favor of their content. The antioxidant activity of wines does not undergoes in any significant changes with drying process and wines produced by drying the raw materials were characterized by a lower lightness. It is necessary to be continued with other trials in way to understand more in detailed the pathway of this component changes with the drying process.

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