RESEARCH ARTICLE **(Open Access)**

Anthocyanin content in dried berry skins and wine produced from dried grapes

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Abstract

Anthocyanin, are the substances which are biosynthesized in the grape skin, extracted during the maceration and vinification and which contribute in wine color. During the aging of wines these substances are converted in their derivates, contributing in wine quality. For this reasons in nowadays there is a big attention of studies in these components. Wines from two grapes variety, a French variety, Cabernet Sauvignon and the other an autochthonous variety of Albania Kallmet were observed. The role of drying in concentration of anthocyanin in skins berry and if this technique is reflected in wine produced from this dried grape, was studied. The results shown that the quantity of anthocyanins from dried skins varied from 136 mg/100gr fresh product to 468 mg/100gr. The dried skins had the higher quantity, but this result was more significant for Cabernet Sauvignon wine than Kallmet wine. Measurement of phenolic content showed no significant changes between wines from dried and non-dried grapes.

Keywords: drying, grapes skin, anthocyanin content, wine.

1. Introduction

Nowadays free radicals have been a big concern for human health as the first synthesizer of potential cancer cells. Their ability to attack different part of cells leading irreversible malfunctions in cases of affected DNA. The damage of the tissue, proteins, unsaturated lipids, carbohydrates and nucleic acid oxidative degradations come from the reactions that free radicals have with O2 [1].

For this reason many diets are based on foods with high levels of antioxidants. These antioxidative substances are also contained in wine and play a great role in protecting the wine during the aging, thereby increasing their overall quality.

Wine is rich in antioxidative substances such as phenols. The minimal concentration of phenolic compounds in commercially produced red wines is rarely below 2.5g/l. Two main groups of phenols occur in wines: flavonoides and nonflavanoides. The most common phenols in white and red wine are flavonoides, catechins and anthocyanidins, but anthocyanidins are only contained in red wine. They originate almost exclusively from the skins and their extraction during the maceration[2].

Anthocyanins are the most present class of flavonoides in grape berries and they possess some roles in biological activities as protection against solar exposure and ultraviolet radiation, free radical scavenging and antioxidative capacity, defense against many different pathogens, attraction of predators for seed dispersal. The glucose is typically sugar in grapes, and their molecules can be linked with the anthocyanidin through bonds at the C3 positions to form the 3-0-monoglycoside anthocyanin as cyanidine [3]

Other studies have shown that the quantities of anthocyanin in postharvest drying depend on the temperature and weight loss of the grape. The best temperature for maintaining the quantity of anthocyanin was 10º C which was sufficient until the weight loss reached 40 % [4].

From comparing the literature the effort of most studies are to find the best conditions and varieties of which the quantity of anthocyanin extracted are higher [5; 6; 7].

The aim of this study is to discover whether drying of skins a good way to extract a high level of anthocyanin compared to not drying skins and if the variety is a factor included in the level of extraction.

2. Material and Methods

2.1. Mechanical analysis of grape

The mechanical analyses of the grapes were done by randomly choosing the clusters for each variety.

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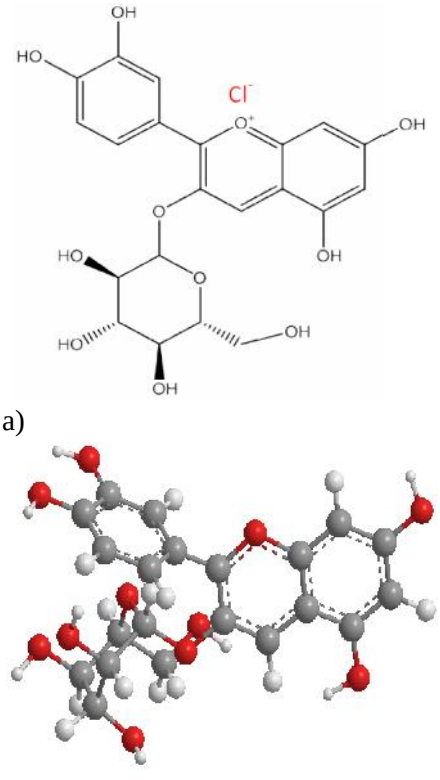
(Accepted for publication 20 June 2014)
***ISSN**: 2218-2020, © Agricultural University of Tirana*

Afterward, the analysis was conducted as described by Kambo [8]

2.2. Sample preparation

Grapes skins were from the two varieties Cabernet Sauvignon and Kallmet in full maturity and obtained in two ways: parts of them were obtained from fresh berries of two varieties and then have been dried for two days and others were obtained from dried berries preliminarily of Cabernet Sauvignon and then were re-dried for two other days. Two important parameters measured for these grapes were: sugar content (obtained with a refractometer) and total acidity (obtained by titration with NaOH). The results were 22.7% (fresh berries) and 27% (dried berries) for Cabernet, 5.77 g/l tartaric acid and for the Kallmet were 25% and 4.95 g/l tartaric acid. Later, 5 g of skin grapes were extracted with 200 ml of solvent (ethanol 20%+ citric acid 0.1M). After 24 hours 200 ml was extracted and then the extract was diluted with 2 buffer solution at pH = 1.0 and pH= 4.5 [9].

For wine samples is not done the dilution with solvent (ethanol 20%+ citric acid 0.1M) because the wine itself has a ethanol content 16-18 % vol so is a medium solvent. After 200 ml the wine samples were taken and diluted with two buffers solution at pH =1.0 and pH= 4.5



a)

b)

Figure 1. Two different views of cyanidin-3-glycoside structure $C_{21}H_{21}O_{11}Cl$. a) two dimensional and b) three dimensional. Red balls represent oxygen atoms, grey balls represent carbon atoms and white balls represent hydrogen atoms.

2.3. Spectrophotomectrical analysis of total anthocyanidins

For a better extraction the skins were left with buffer solution for one hour and then measured with spectrophotometer VIS (SPECORD version 2.3, WinASPECT) in two wave length, 520 nm and 700 nm. Content of anthocyanins is expressed as cyanidin-3-glucoside equivalent in mg/100 gr fresh product.

The total absorbance of diluted sample was calculated as follows:

$A_{(\text{total absorbance})}$ =[(A520- A700)pH1 - (A520- A700) pH4.5]

Total anthocyanin content was represented as cyanidin-3- glycoside and expressed as mg/100gr of fresh product. Calculated as follows:

$$C = \frac{A * Mw * Df * Vfl * 100}{\varepsilon * l * ms}$$

Were the molecular weight (Mw) of cyanidin-3-glucozid is 449.2, Diluation factor (Df) that is 10, mass of sample (Ms) that is 5 gr for skins analyses and for wine samples this variable is calculated with the percentage of skins that could be found in 200ml wine in compliance with mechanical analyses Table 1. The Ms for each variety was 63.2 gr for Kallmet and 100.8 gr for Cabernet, the Vfl volumetric flask wich was brought the extract and it is 200ml, Ɛ molar absorptivity = 25740 , l path length of the cyvet that is 1cm[10].

2.4. Phenolic content

The Wine Phenolic content like anthocyanin, short polymeric pigments, total polymeric pigments, tannins, iron reactive phenols and non-tannin iron reactive phenols were estimated according to Skogerson-Boulton Model [11].

2.5. Statistical analysis

ANOVA one-way to determine statistical significance between sample populations at the $P < 0.05$ confidence level were conducted with R version 3.0.2 (EIC, Switzerland).

3. Results and Discussion

Table 1 shows the mechanical analysis of grapes from two varieties, which is important for understanding the variety and productivity that is expected. From the table, we see that Kallmet had a higher theoretical productivity that is twice higher than Cabernet Sauvignon. This is connected directly with the pulp-content, which was almost twice as high as well. From the percentage of skin content, the

expectations were that Kallmet should have a lower content in anthocyanin. It skin quantity was lower than in Cabernet Sauvignon and the skin is the major contributor of anthocyanin in wine.

Anthocyanin undergoes in reversible structural transformation when there is a change in pH and this occurrence is closely linked with different absorbance spectra. At a pH of 1 predominate the color is the oxonium form and in a pH of 4.5 the colorless hemiketal is the predominate form. These methods of pH differential allow for the total amount of anthocyanin to be regarded [9]. As the results show, the highest level of anthocyanin belongs to the grape skins that have been re-dried (Figure 2).

So there are two possible factors that affect the quantity of anthocyanin in samples: temperature and exposure to the light.

Table 1. The mechanical analysis of grapes from two varieties

	Cluster gr	**Stem** %	Berries %	Weight of 100 berries	**Skin** %	**Pulp** %	**Seed** %	Construction ratio	Theoretical productivity
Cabernet	191.9	2.93	80.3	127.6	25.2	43.8	5.16	27.4	35.17
Kallmet	196.16	2.4	96.65	278.9	15.8	74.7	4.32	40.1	72.15

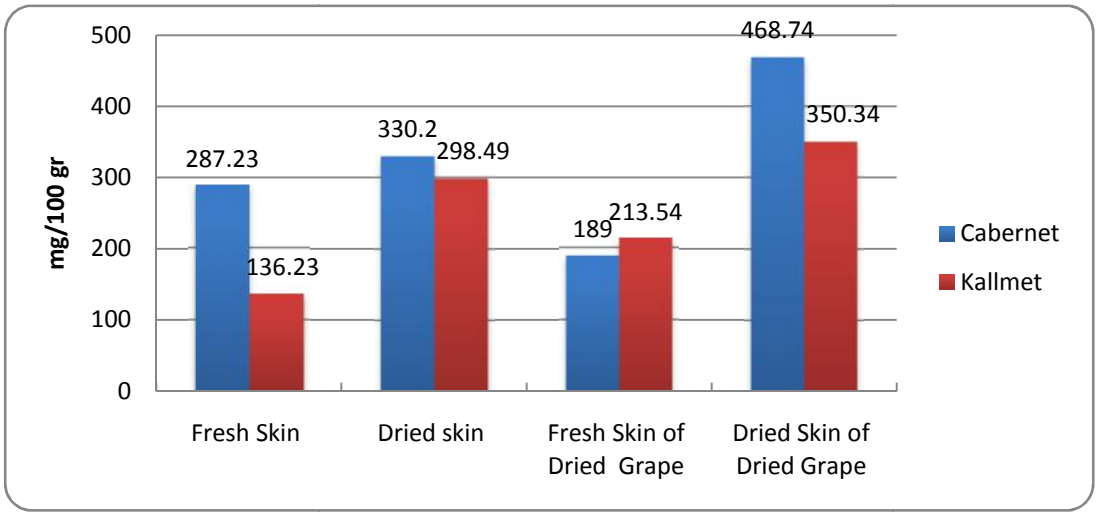


Figure 1. Comparative diagram showing the anthocyanin content in dried skin berry and non-dried skin berry

The temperature is a very important factor in anthocyanin synthesis. At lower temperatures around 25° C there is an increase in the synthesis of anthocyanin and at high temperatures around 35° C there is a degradation of anthocyanin and inhibition of their accumulation. This explains the fact that during the warmest years the level of anthocyanin are lower than in a cooler years[3]. This is related to the fact that re-dried grape skins had the highest level of total anthocyanin, because the temperature of both dried, of the grapes initially and of skins, does not exceed the 30 ° C a good temperature for a better level of anthocyanin.

Another component being affected from temperature is the concentration of sugar. Sugar is a chemical factor that regulates the anthocyanin biosynthesis in grapes. During the ripening the level of total sugar in grape skin increase and the accumulation of anthocyanins usually happens one week later than the massive increasing of sugar content, showing the closed relationship of sugar in anthocyanin biosynthesis[3].

The other key factor is light exposure, which the samples have passed under during the drying treatment has a great role in anthocyanin content. Light exposure has a positive effect on collection anthocyanin concentration and shading can reduce their concentration. This is related with the reduction of genes that are important in anthocyanin biosynthesis [3].

At veraison the major pigments are peonidin-, malvidin-, and cyanidin-3-glucosides and towards maturity the most abundant became malvidin- and delphinidin-3-glucosides. Accumulation of cyanidin-

3-glucoside is mostly influenced by prevailing environmental conditions. Accumulation of phenolics during veraison is restricted at low light intensity. In the berry skin, after the removal of the shade cloth, had a partially restored phenolic accumulation [12].

The phenolic content also as showed in Tab. 2 shows that wines from dried Kallmet had a lower quantity of anthocyanins and tannins compared with wines from non-dried grapes and for the Cabernet Sauvignon the trend was not the same. But in general, were not significant changes in anthocyanin content in the different samples. The wines from dried grape had a higher content of these compounds than the wines from non-dried grapes. The fact that this response was not the same for both varieties may stem from the mechanical changes that occur in grape skins during drying process. Different studies show that during drying the skin of the grapes tend to become harder and thicker. These factors play an important role in extraction of anthocyanin during the maceration, because the harder and thicker the skin the more it is difficult to extract all desired phenolic compounds during the time of maceration [13]

For determination of anthocyanin accumulation in Cabernet Sauvignon, light is not the only factor. Light is important for holding maximum activity of enzymes involved in the production of these compounds, always after anthocyanin synthesis begins, bunch shading after veraison reduced anthocyanin synthesis. Also, bunch shading delayed ripening and reduced anthocyanin concentration. The anthocyanin pattern changed significantly if the light conditions changed after veraison. In the last stage of ripening, the light exclusion caused esterification processes that led to the production of the acetate form.

The importance of grape skin consistency was reinforced by the results of anthocyanin content in wine produced from these dried grapes Figure 3. The content of anthocyanin was much higher in wines produced from the dried Cabernet variety.

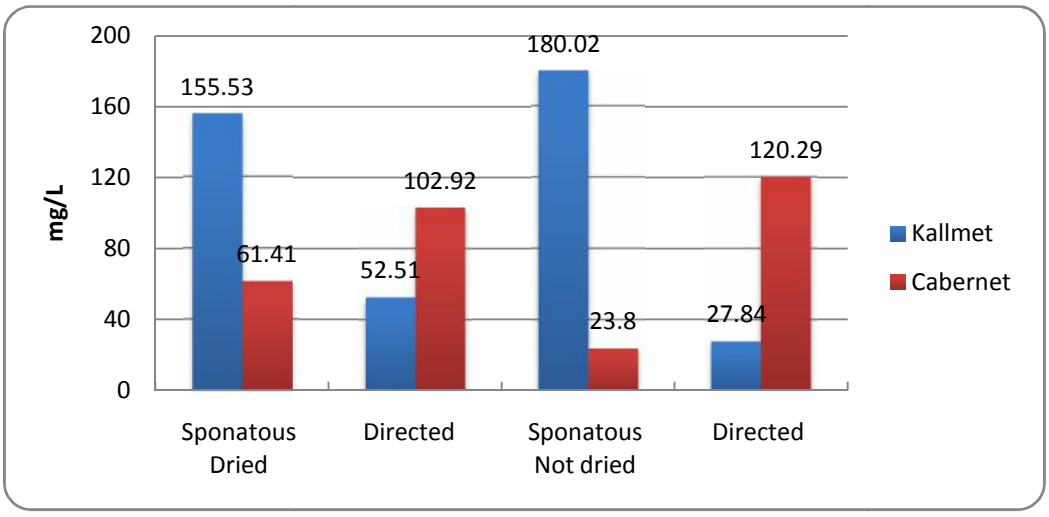


Figure 2. Comparative diagram showing the anthocyanin content in wines produced from two varieties, dried and non-dried grapes.

Table 2. Phenolic Content in wine

	KALLMET Not Dried Spontaneous		KALLMET Not Dried Directed		KALLMET Dried Directed		KALLMET Dried Spontaneous		CABERNET SAUVIGNON Not Dried Directed		CABERNET SAUVIGNON Not Dried Spontaneous		CABERNET SAUVIGNON Dried Spontaneous		CABERNET SAUVIGNON Dried Directed	
	Mean*	S.D	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D
.Anthocyanins (mg/L)	588^{a**}	18	734^{a}	25	618^{a}	79.6	802^{a}	435	568^{a}	313	305^{a}	24.8	824^{a}	172	681^{a}	173.8
Tannins (mg/L)	1179^{ab}	35.1	1370^{b}	22	1116^{ab}	64.3	1007^{ab}	57.2	1137^{ab}	422	772^{a}	21.5	1351^{b}	88.5	1147^{ab}	35.92
Total Iron Reactive Phenols (mg/L)	1776^{ab}	128	2160^{b}	95	1478^{ab}	344	919^{a}	810	1223^{ab}	238	1103^{ab}	87.6	1166^{ab}	280	1208^{ab}	523.1
Polymeric Pigments	2.42^{ab}	0.39	2.55^{ac}	0.3	2.83^{ac}	0.98	1.59^{a}	1.41	4.29^{bc}	0.79	4.08^{ac}	0.04	5.13^{c}	0.60	4.25^{ac}	1.72
Small Polymeric Pigments (AU)	0.00^{a}	0.00	0.00^{a}	0.0	0.26^{ab}	0.44	0.13^{a}	0.23	1.40^{b}	0.25	1.39^{b}	0.38	0.28^{ab}	0.49	0.95^{ab}	0.83
Large Polymeric Pigments (AU)	2.42^{a}	0.39	2.55^{a}	0.3	2.57^{a}	0.67	1.46^{a}	1.27	2.89^{ab}	1.03	2.69^{a}	0.34	4.85^{b}	0.22	3.29^{ab}	0.89
Non-Tannin Phenols (mg/L)	1337^{ab}	103.5	1600^{b}	80	1034^{ab}	266	657^{a}	580	552.8^{a}	479	871^{ab}	63.9	811^{ab}	212	842^{ab}	402.6

*The determination was performed in triplicate (n=3) and value are given as mean.

**Different letters indicate significant differences between samples performed with ANOVA one-factorial ($P<0.05$), Tukey test.

4. Conclusions

Anthocyanin are a very important substance of grapes that during maceration passed to the wine and act then like a very strong antioxidant and in this way protect the wine during aging. For this is a very great attention from scientist to find a way for a good extraction of them or to find better environmental conditions for a better anthocyanins biosynthesis in grape berry. There is a lot of information in nowadays about the way of biosynthesis of anthocyanins in berry, about the necessary condition but also there is some of the question that doesn't have answer in this direction. However, the re-dried berry skins remaining longer in bunch had better anthocyanin content than if they that were not re-dried. The important factors affecting this result were the temperature and the light. But others factors that can be taken in account remain to investigate in the future. Drying the grapes, result in small changes in wines from both Kallmet and Cabernet Sauvignon varieties.

5. Acknowledgements

The authors would like to thank the staff of Changins Engineering School, University of Applied Sciences of Western Switzerland, for their help in this research.

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