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Phenolic Compounds of Grape Varieties Grown in the Northern Temperate Climate

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Abstract – **This study investigated the influence of Northern cool climate and latitudes on phenolic compounds of black hybrid grape varieties. The concentrations of phenolic compounds in different grape varieties Zilga and Nordica, grown at latitude of 60°24' Nord were analyzed and compared them to those in Spanish variety Autumn Royale grown 37°59' Nord. Changes in sugar and phenolic concentrations during grape development were followed in Nordica. The influence of latitudes on the phenolic compounds was investigated in Rondo grown at the latitudes of 55°31' to 61°20' Nord. The phenolic compounds were analyzed by HPLC. The sugar concentrations and content of almost all phenolic compounds were distinctly higher in Zilga and Nordica than in imported table grape Autumn Royale. No systematic differences in the phenolic contents between latitudes were found for Rondo. This study shows that the phenolic compounds and sugar concentrations of different black grape varieties grown at the Northern latitudes were considerably different from that of the imported Southern grape. Thanks to the climate change scenarios and grape breeding it might be possible to grow high quality grapes in Southern Scandinavia after a few decades.**

Keywords – **Wine Growing, Cool Climate, Climate Change, Resveratrol, Effect Of Latitude.**

I. Introduction

Phenolic compounds are divided into flavonoid and non-flavonoid groups. So far, the structures of 8.000 phenolic compounds have been deciphered. Over 4.000 of them are flavonoids [1]**.** They protect plants from many stressful elements in their environment, such as infections, heat, cold, aridity, moisture, and ultraviolet light [2] [3]. In addition, many phenolic compounds have properties which are beneficial for human health and diseases preventions, but their exact action mechanisms are partly so far unknown. Grapes and wine are well-known sources of various phenolic compounds [4].

Many experimental studies have shown that ample light has increases phenolic compound and anthocyanin concentrations in the skin and pulp of grape berries [5] [6], but scant light decreases them [7]. High phenolic compound concentrations have been measured in grapes from the Bodega Colome's vineyards which are 2.300 to 3.111 m above the sea level, in the city of Salta in Argentina. The high concentrations have been attributed to the growing season's coolness, the high temperature difference during day and night, and ample ultraviolet light (www.bodegacolome.com). Also, in Northern Europe the long day during growing season and the mild stress created by the cool climate might increase phenolic compounds of grapes.

Because of global warming, viticulture is expanding to Northern Europe: to the area around Copenhagen (55º33´ Nord), to the area around Stockholm (59º10´ Nord), to the area near Helsinki (60º10´ Nord), and to the Finnish west coast up to 63° Nord. According to moderate RCP (Representative Concentration Pathways) scenarios, the mean annual temperature in Northern Europe could rise 2 to 3°C by the year 2100 [8]. This means that temperature in Northern Europe could be the same as currently in Central Europe. As a result, viticulture will spread further north where the longer day and ample light during growing season will affect grape's properties and phenolic concentrations.

The effects of the northern environmental conditions on the phenolic compound concentrations have been studied in wild berries, such as bilberries (*Vaccinium myrtillus* L.). In bilberries, which have adapted to grown in the 69th latitude, anthocyanin levels are more than twice higher than those in bilberries which grew in the 54th latitude [9] [10]. No similar studies have been reported for grape phenolic compounds.

The aim of this work was to analyze the concentrations of phenolic compounds in black hybrid varieties Zilga and Nordica, grown in Southern Finland, and to compare them to those in Autumn Royale grown in Spain in order to examine the quality of this plenty into Finland imported table grape variety. In addition, changes in sugar and phenolic concentrations during grape development were followed in Nordica during the last month before the harvest. The influence of the hardiness zone on the phenolic compounds was investigated in Rondo grown in the different Nordic latitudes.

II. Material and Methods

The phenolic compounds in the four black hybrid varieties: Zilga, Nordica, Rondo and Autumn Royale were analyzed with HPLC methods described by Kumsta et al. [11]. The following compounds were analyzed from the juice: gallic acid, caffeic acid, caftaric acid, *p*-coumaric acid, ferulic acid, *cis*-resveratrol, *trans*-resveratrol, *cis*-piceid, *trans*-piceid, (+)-catechin, (–)-epicatechin, quercetin, myricedin and anthocyanins. The hybrid varieties Zilga *(Dvietes 4–2–08) (Smuglyanka x Dvietes Zila) x Jubileinaja Novgoroda)* and Nordica (*V. vinifera x V. labrusca*) had been grown in the open air in Southern Finland. The hybrid variety Rondo *((Précoce de Maligre x V. amurensis Rupr.) x St Laurent)* had been grown in Southern Finland (Pälkäne and Tuusula), and in Southern Sweden (Klagshamn). The hybrid variety Autumn Royale

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(*Autum Black x C74-1*) was a table grape grown in Spain and exported to Finland. The growing conditions and sampling times are listed in Tables 1 and 2.

Table 1: Wine growing localities

Country	Locality	Latitude Altitude	Above sea level (m)	Hardiness zone[a]	Average daily temp. (°C)[b]	Solar irridation (Wh/m^a)[b]	Longest day (h:min)[b]
Finland	Pälkäne	61°20'Nord 24°16'East	93	4	11.8	861	19:26
Finland	Tuusula	60°24'Nord 25°01'Eeast	63	5	13.2	879	19:02
Sweden	Klagshamn	55°31'Nord 12°56'East	12	7	13.8	957	17:31
Spain	Murcia	37°59'Nord 01°07'East	153	10	22.8	1228	14:46

[a]www.gardenweb.com/zones/europe/ [b]April 1 to September 30

Table 2: Grape varieties and sampling times

Community	Grape variety	E-L[a]	Sampling times (year 2013)	Berry weight Mean±SD[c] (g)
Tuusula	Zilga	37	September 7	1.7±0.1
Tuusula	Nordica	34	August 7	1.7±0.1
Tuusula	Nordica	35	August 18	1.7±0.1
Tuusula	Nordica	36	August 28	1.7±0.1
Tuusula	Nordica	37	September 7	1.8±0.2
Pälkäne	Rondo	37	September 7	1.8±0.1
Tuusula	Rondo	37	September 7	1.8±0.1
Klagshamn	Rondo	37	October 2	1.8±0.2
Espanja	Autumn Royal	37	October 3[b]	8.5±0.2

[a]Eichhorn-Lorenz, [b]purchase date in Finland, [c]n =

The sugar concentrations (°Brix) in the grape samples were measured with refractometer (HR-180; Optika, Ponteranica, Italy) from the juice of twenty grape berries individually. After determining the phase of the grapes' development according to Eichhorn-Lorenz number [12], collecting and weighing them, and determining the sugar concentration the samples were frozen and stored at -25°C until they were analyzed with HPLC [11]. The concentrations of phenolic compounds were measured from the juice of ripe grape berries using two parallel samples of 40 grape berries.

III. Results and Discussion

The sugar concentrations in the juice of ripe grape berries Zilga and Nordica grown in Southern Finland (60°24' Nord) were 17.9 and 17.4 g/L and in the Spanish table grape Autumn Royale 15.8 g/L. The sugar concentration of ripe berries is between 15 and 25°Brix [13]. The smaller sugar concentration in Autumn Royale could be explained by the fact that the grapes were picked before they were ripe because they needed to be stored and transported for a long time for export.

3.1. Phenolic acids

Table 3: Concentrations of sugar and phenolic compounds of grape varieties grown in Southern Finland and Spain

	Grape variety	Zilga Nordica	Autumn Royale
Sugar Mean±SD	g/L17.9±0.8	17.4±1.4	15.8±0.4
Phenolic compounds Mean (min – max) mg/L			
gallicacid	41.5 38.5 – 43.6	49.5 44.2 – 54.8	0.8 0.6 – 1.0
caffeicacid	84.1 81.0 – 87.2	18.5 16.0 – 20.9	4.9 4.5 – 5.2
caftaric acid	76.2 78.8 – 73.5	10.0 7.7 – 12.3	0.7 0.6 – 0.7
p-coumaric acid	7.0 5.6 – 8.4	6.6 6.1 – 7.1	0.3 0.2 – 0.3

ferulic acid	2.7 2.3 – 3.0	1.1 1.0 – 1.2	0.4 0.3 – 0.4
cis-resveratrol	1.8 1.7 – 1.8	1.7 1.1 – 2.2	0.4 0.4 – 0.4
trans-resveratrol	14.8 14.1 – 15.4	3.0 1.9 – 4.1	0.2 0.2 – 0.2
cis-piceid	1.7 1.1 – 2.3	2.3 2.1 – 2.5	1.2 1.0 – 1.4
trans-piceid	1.7 1.1 – 2.3	1.3 1.2 – 1.3	0.2 0.2 – 0.2
(+)-catechin	704.9 700.9 – 708.9	973.1 956.5 – 989.7	10.7 7.4 – 14.00
(–)-epicatechin	1339.6 1277.7 – 1401.5	1297.5 1267.3 – 1327.7	2.5 1.2 – 3.8
quercetin	2.5 1.2 – 3.7	0.3 0.3 – 0.3	0.6 0.4 – 0.8
myricetin	0.1 0.0 – 0.2	0.02 0.00 – 0.04	0.00 0.00 – 0.00
anthocyanins	1281.6 1232.0 – 1331.2	608.8 582.4 – 635.2	302.8 300.8 – 304.8

The gallic acid means were close to each other in two parallel measurements of Zilga and Nordica (41.5 and 49.5 mg/L) but the gallic acid concentration in Autumn Royale was only 0.8 mg/L.

The gallic acid concentrations in Zilga and Nordica were higher than those reported for grapes in previous studies [14]. The gallic acid concentration of Autumn Royale was distinctly smaller. It was close to concentrations measured in green grapes [14]. The difference in the hydroxycinnamate concentrations in Zilga and Nordica were most obvious in the caffeic and caftaric acid concentrations. The caffeic acid concentration in Zilga was 4.5 times higher than in Nordica and the caftaric acid concentration was eight times higher than in Nordica. The caffeic acid concentration in Zilga was similar to the caffeic acid concentrations measured earlier [15] in several grape varieties.

The *p*-coumaric acid values in Zilga and Nordica were close to each other (Table 3) and significantly higher than in previous studies [15]. The ferulic acid concentration in Zilga was more than twice higher than in Vitis 'Nordica' (2.7 mg/L and 1.1 mg/L). Compared to earlier results [16], the *p*-coumaric acid values in Zilga and Nordica were similar to the European-Asian hybrid grapevines and North American grapevines but Zilga's and Nordica's ferulic acid concentrations were lower. The caftaric and *p*-coumaric acid concentrations in Autumn Royale were lower than 1 mg/L and below the earlier measurements results.

3.2. Stilbenes

The resveratrol values of Zilga and Nordica were as high or higher than in earlier studies [17]. The *trans*-resveratrol in Zilga (14.8 mg/L) was exceptionally high (Table 3) and higher than in earlier measurements from dark grapes [18]. The *trans*-resveratrol in Nordica (3.0 mg/L) was also rather high. The *cis*-resveratrol in Autumn Royale (0.4 mg/L) was within the concentrations measured earlier [18]. The *trans*-piceid and *cis*-piceid values in the Vitis 'Zilga' and 'Nordica' grapes (1 to 2 mg/L) were lower than previously measured in red Brazilian wines [19]. The stilbenes in Autumn Royale were lower than 1 mg/L except the *cis*-piceid (1.2 mg/L) was close to Zilga (Table 3).

3.3. Flavonoids

The (+)-catechin and (–)-epicatechin concentrations in Zilga and Nordica were plenty higher when compared to previous measurements in black grapes [20]**.** The (+)-catechin concentration in Zilga was 28 % smaller than in Nordica but the (–)-epicatechin concentrations in Zilga and Nordica differed from each other only 3 %. In these cultivated grape varieties they were similar to the concentrations in wild sea-buckthorn and bog bilberry [21]. The (+)-cathecin and (–)-epicatechin concentrations in Autumn Royale were very low compared to those in Zilga and Nordica and they did not reach values which had been measured earlier in black grapes [22].

In all varieties, the flavonol (quercetin and myricetin) concentrations were lower than concentrations which had been measured earlier in black grapes [15]. Anthocyanins were the major phenolic compounds in Zilga, Nordica and Rondo. In Zilga the anthocyanin concentration was more than two times higher than in Nordica and four times higher than in Autumn Royale (Table 3). It is usual to see such great differences in the phenolic compounds between grape varieties [16] [22].

3.4. Effect of development stage

During the 30 days before harvest, the anthocyanin concentration increased rapidly in Nordica while the flavan-3-ol concentration remained stable (Table 4). Similarly, the concentration of *trans*-resveratrol increased during the last month but such clear change was not observed for *cis*-resveratrol. The caftaric and caffeic acids fluctuated but the ferulic acid stayed the same.

Table 4: Changes of sugar content and phenolic compounds of 'Nordica' grapes during the last month before harvest

Date	sugar g/L	anthocy-anins mg/L	flavan-3-ols mg/L	*trans*-resveratrol mg/L	*cis*-resveratrol mg/L	caftaric acid mg/L	caffeic acid mg/L	ferulic acid mg/L
Aug. 7	10.3	52.3	4102.3	0.5	1.7	6.4	12.6	1.4
Aug. 18	13.6	91.2	4312.5	0.6	1.2	3.6	16.6	1.6
Aug. 28	15.5	164.0	4203.2	1.5	1.3	7.0	10.5	1.5
Sept. 9	17.4	608.8	4326.7	3.0	1.7	10.0	18.5	1.1

3.4. Effect of latitude

Grapevine is a new agricultural plant above the latitude of 55°N. The northern climate and growth conditions different from southern climate and growth conditions have been observed to influence on the growth, development and phenolic compounds of wild berries [9] [10], and therefore the influence of different Nordic latitudes on phenolic compounds of grapes was investigated by comparing the phenolic compounds of Rondo grapes grown at latitudes from 55°Nord to 61°Nord. The linear distance between 55°N and 61°N was 670 km. No systematic effects of the latitude on the phenolic compounds of the same variety could be observed (Table 5). However, the (–)-epicatechin, and (+)-epicatechin contents of Rondo grapes, grown at the latitudes of 60 to 61°Nord, were 1.5 to 4 fold compared to Rondo grapes grown at latitude of 55°N, but the anthocyanins of Rondo grown at the southern latitude were higher.

Table 5: Concentrations of sugar and phenolic compounds of Rondo grapes at different latitudes

Grape varieties	Rondo (Pälkäne)	Rondo (Tuusula)	Rondo (Klagshamn)
Sugar Mean ± SD	g/L17.8±1.0	17.4±1.0	18.2±0.7
Phenolic compounds Mean (min – max) mg/L			
gallicacid	66.4 59.9 – 72.9	96.8 70.2 – 123.4	34.8 33.5 – 36.0
caffeicacid	85.8 68.5 – 103.0	16.3 14.4 – 18.2	27.7 17.6 – 37.7
caftaric acid	66.7 60.4 – 72.9	11.8 10.0 – 13.5	21.3 10.8 – 31.8
p-coumaric acid	4.0 3.2 – 4.8	7.3 6.4 – 8.1	3.5 2.3 – 4.7
ferulic acid	0.8 0.8 – 0.8	2.7 2.5 – 2.8	1.6 1.0 – 2.2
cis-resveratrol	1.2 1.2 – 1.2	1.8 0.3 – 3.4	4.9 4.2 – 5.6
trans-resveratrol	1.2 0.8 – 1.5	10.3 5.2 – 15.4	2.3 2.0 – 2.6
cis-piceid	4.2 3.7 – 4.6	2.1 1.7 – 2.5	4.3 3.9 – 4.7
trans-piceid	0.8 0.7 – 0.8	1.20 1.0 – 1.4	2.3 1.9 – 2.7
(+)-catechin	864.9 862.6 – 867.2	696.3 655.1 – 737.5	509.9 480.3 – 539.5
(-)-epicatechin	788.7 740.9 – 836.4	1473.4 1310.1 – 1636.6	248.6 295.2 – 202.0
quercetin	0.6 0.5 – 0.7	0.5 0.4 – 0.6	1.9 1.3 – 2.5
myricetin	0.1 0.00 – 0.2	0.4 0.00 – 0.8	0.8 0.7 – 0.8
anthocyanins	3092.8 2763.2–3422.4	1156.8 987.2 – 1326,4	3720.0 3657.6 – 3782.4

3.5. Health effects of phenolic compounds

The flavonoid concentrations in grapes and wines made from grapes have been measured many times, especially after Renaud and deLorgeril presented their so-called French Paradox [4]. According to the French Paradox stilbenes, especially resveratrol, in French red wine can prevent cardiovascular disease. In animal testing (*in vivo*) and laboratory experiments (*in vitro*) it has been discovered that phenolic compounds in grapes and grape seeds are strong antioxidants. It has been suggested that products which contain flavonoids lower the risk of developing cancer, diabetes, obesity, or illness in the central nervous system [23]. However, the latest long term study Semba et al. [24] showed that the regular consumption of resveratrol did not prevent infections or prevent contracting cancer or cardiovascular disease nor did it contribute to longevity.

Analysis from the HPLC measurements and their meta-analyses shows that the concentration of some flavonoids in grapes and wines can be zero or that the differences in the concentrations can be up to thousand-fold [16] [22]. Even the same grape variety can have significantly different flavonoid compound concentrations in different countries and localities depending on altitude and latitude of the growth environment. These differences showed up in this study, as well. Therefore, the 'reference values' for the phenolic compounds of the grapes cannot be determined. Because of the great difference in flavonoid concentrations and because there are an estimated 10.000 grapevine varieties, it is impossible to put the grapes in order of superiority based on flavonoid concentrations, either.

IV. Conclusion

The study used the HPLC method to measure the phenol compound concentrations in three dark hybrid grape varieties (Zilga, Nordica, and Rondo) grown in the north, and compared these to the phenol compound concentrations of hybrid varieties (Autumn Royale) grown in the south and commonly imported to the north as edible table grapes. This showed that the phenolic acid, resveratrol, and flavonoid concentrations of Zilga, Rondo, and Nordica were significantly higher than those of the Autumn Royale. Likewise, their sugar concentrations were clearly higher than that of the Autumn Royale. Based on this sample, it can be concluded that some grapes imported to the north for eating may be of inferior quality. There were no systematic differences between phenol compound concentrations of grapes grown in the north, although they showed somewhat higher *trans*-resveratrol concentrations than dark grapes had earlier. Consequently they may also offer more health benefits. *Trans*-resveratrol values increased strongly for the month preceding the last harvest, so they will be at their highest value only in fully ripened grapes. There were no systematic differences between the phenol compound concentrations of the same grape variety (Rondo) grown in high latitudes (55° to 61° Nord) in a north-south direction, except for the grapes that were grown furthest south (55° Nord), which had the highest anthocyanin contents. To discover the importance of climate, climate change and latitudes to phenolic compounds of grapes, monitoring of several years will be required.

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