Grape Cultivars Classification by Biochemical Constituents and HPLC Profiles

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Summary

The germplasm of grapevines possesses a great degree of variability and can be divided into geographic groups. Recent genetic investigations have validated these classifications, and further divided types into geographic-genetic groups to better describe their similarities and differences. The purpose of this research was to analyze the biochemical components of nine different varieties of fruit and to categorize different types of grapes using multivariate analysis. Grapes include a number of significant secondary metabolites, including polyphenolic chemicals, which influence the overall quality of the fruit as well as its sensory qualities. The fruit extracts of the Sitkan cultivar yielded the greatest total phenolic content of any other cultivar tested. Both the Ranya and the Rost Cultivars had exceptionally high levels of antioxidant activity. The HPLC analysis of natural compounds (including, rosmaric acid, caffeic acid, chlorogenic acid, cinnamic acid, p-coumaric acid, rutin, apigenin, gallic acid and quercetin) showed that chlorogenic acid and gallic acid were the most common phenolic compounds in the grape samples. Caffeic acid was the third most prevalent compound, followed by p-coumaric acid and cinnamic acid. Using multivariate analysis, the various varieties of grapes were categorized into one of three primary groupings. According to the findings, the various cultivars of grapes are the most important source of antioxidants and other useful phytochemical components. According to the findings of HPLC testing, the Alan cultivar contained a significant amount of phytochemical components.

Key words

antioxidant, gallic acid, chlorogenic acid, *Vitis vinifera*, phenolic compounds

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Introduction

The fruits quality depends mostly on the quality of the grapes that are used in production (Gambetta et al., 2016). The concentrations of free and bound volatile aroma components, titratable acidity (TA), total soluble solids (TSS), amino acids, pH, polyphenols, required yeast nutrition and other chemicals all have an impact on the final sensory profile of the wine that is produced. Quality is connected to grape chemical composition, affecting the resulting sensory profile of the wine (Dennis et al., 2012; Gambetta et al., 2014).

The assessment of grape quality is crucial because it affects the distribution of fruit products, viticultural methods, and producer payments per ton of fruit. Historical parcel records, fundamental chemical measurements (TA, TSS, and pH), berry tasting and yield are currently the most often used parameters to assess the quality of Chardonnay grapes in Australia (Longbottom et al., 2013; Gambetta et al., 2016). Although these criteria offer valuable information, their application as independent, unbiased quality measurements has some drawbacks. In addition, quality and the presence of anthocyanins and polyphenols have been clearly linked in red cultivars (Ristic et al., 2016; Niimi et al., 2018); there are no such linkages between white variants and any particular category of chemicals. Environmental factors like soil type, solar exposure, altitude, and climate have all been shown to influence the grape quality and composition (Alessandrini et al., 2017). As a result, specific vineyard settings have been identified as being more suited to a variety of wine grape varietals.

Understanding the grape extraction chemical content is greatly important throughout enology. The number of studies dealing with this topic has increased steadily in recent years. However, these well-known chemometric approaches are now used to evaluate different processes in wine production (Revilla and González-SanJosé, 2002). One of the most important applications is the differentiation and classification of wine samples in terms of their chemical composition as well as some characteristics such as geographical origin, quality or variety (cultivar). Biogenic amines together with amino acids (Héberger et al., 2003) and phenolic data (Csomós et al., 2002) have been used to differentiate Hungarian white and red grapes, while Spanish grape varieties have been differentiated based on volatile composition and classical parameters (Aleixandre et al., 2002). Sensory data are used to classify grapes according to their organoleptic profile (De La Presa-Owens and Noble, 1995). However, according to various studies, better differentiation of wines based on vintage or type and geographical origin is achieved (Nogueira and Nascimento, 1999) when physicochemical data are used instead of sensory descriptors. Polyphenols are another group of compounds that have been effective in distinguishing wines. Polyphenolates have been shown to be more beneficial than amino acids, volatile and classical data in identifying Spanish wines by grape variety (De La Presa-Owens et al., 1995).

It is therefore clear that chemical data and chemometric methods are important tools for the classification of grapes. In the present study, nine different grape varieties were studied in detail in the Kurdistan Region, Iraq. It is expected this can provide important data and new knowledge valuable for the development of breeding strategies and the selection of varieties with higher phenolic content for the synthesis of natural antioxidants for pharmaceutical and food products.

The aim of this study was to establish a classification model to classify the grape varieties of Kurdistan based on their phenolic composition using chemometric approaches, independent of the effects of geographical origin and climate.

Material and Methods

Grape Samples

The four-kilogram grapes of the *Vitis vinifera* L. cv variety were hand-picked when they reached the desired level of ripeness for trade (21.6 °Brix) in the nine geographical indications of the vineyards of the Kurdistan region (see Table 1). The samples were placed on ice, stored at a temperature of -20 degrees Celsius until needed, and then destemmed while frozen.

Polyphenols

The Process of Extracting Polyphenols

After removing 2 grams of powdered sample and adding 4 milliliters of methanol solvent containing 1% acetic acid, the remainder of the sample was extracted using ultrasonic waves for a period of 20 minutes. This was done so that polyphenols could be obtained. An HPLC (high-performance liquid chromatography) device model 1100 series (Agilent Technologies, USA) was used to isolate, identify, and quantify the phenolic acids to be investigated in this study. This apparatus had a column oven with a temperature of 25 °C, a degassing system, a four-solvent gradient pump, an injection loop with a capacity of 20 µL, a diode array detector with wavelengths of 250, 272, and 310 nm, respectively, and a degassing system. Mainsch of Germany carried out the procedure of isolating the substance on a Ceylon octadecyl column (with an inner diameter of 4.6 mm, a length of 25 cm, and a particle size of 5 µm ZORBAX Eclipse XDB). In order to perform the data analysis, the Chemstation program was used.

Total Antioxidant

Using DPPH as the measurement standard, the total antioxidant was determined according to Brand - Williams et al. (1995).

Total Phenolic Content

The Folin-Cicalteau method, as described by Singleton et al. (1999), was used to measure the total phenolics content of the sample. Some minor adjustments were made to the procedure in order to account for the colorimetric oxidation/reduction reaction of the phenols. In order to extract the polyphenols, a fine powder of 1 g of floret was mixed with 10 mL of methanol at 85% concentration. Following the addition of 2.5 mL of Folin-Cicalteau reagent and 2 mL of 7.5% sodium carbonate to 250 µL of extract, another 250 µL of sterile distilled water were added to the mixture. Between one and two hours were spent shaking the samples. Utilizing a spectrophotometer, the absorbance of the samples was evaluated to be 765 nm (PG Instruments T80 UV, UK). The standard curve was constructed with gallic acid as the solvent. The findings were reported in terms of mg GAE 100 g^{-1} fresh weight.

Table 1. The grape samples evaluated in this study are organized geographically

Cultivar	Province	Height (m)	latitude	longitude
Alana	Erbil	821 m	36.5733	44.4424
Ranya	Sulaimaniya	578 m	36.3513	44.7697
SidakanR	Erbil	1002 m	36.7993	44.6705
Malakan	Erbil	1452 m	36.4988	44.5298
Rost	Erbil	1478 m	36.7094	447617
SidakanT	Erbil	1002 m	36.7993	44.6705
Balisan	Sulaimaniya	889 m	36.4070	44.5776
Sitkan	Erbil	1178 m	36.6908	43.9137
Dohuk	Dohuk	526 m	36.8608	42.9975

Statistical Analyses

SAS software was used to conduct the analysis (SAS 9.2, SAS Institute, Cary, NC). To determine whether there were statistically significant differences between the groups, one-way analysis (ANOVA) was combined with Tukey HSD test at a significance of $P \leq 0.05$. In addition, principal component analysis (PCA) and hierarchical cluster analysis (HCA) were performed on the data analysed with XLSTAT to distinguish the different genotypes by phytochemical compounds.

Results

HPLC Analysis of the Samples

Fig. 1 shows the chromatograms of nine different standards passed through an HPLC. The amounts of some phenolic compounds (caffeic acid, gallic acid, chlorogenic acid, cinnamic acid, p-coumaric acid and rosmarinic acid) and three flavonoids (quercetin, rutin and apigenin) varied significantly between the nine cultivars analysed in this work (Table 2). Chlorogenic acid and gallic acid were observed as the most abundant phenolic compounds in the grape extracts. The higher concentrations of caffeic acid (19.07 mg kg^{-1}), gallic acid (763.62 mg kg^{-1}), chlorogenic acid(988.36 mg kg^{-1}), chlorogenic acid (988.36 mg kg^{-1}), p-coumaric acid (107.51 mg kg^{-1}), cinnamic acid (2.34 mg kg^{-1}), rutin (3.58 mg kg^{-1}), apigenin (23.04 mg kg^{-1}), rosmarinic acid (36.32 mg kg^{-1}) and quercetin (52.98 mg kg^{-1}) were observed in the fruit extracts of Alan, Alan, SidakanR, Alan, Alan, Dohuk and Sitkan grapes. The highest concentrations of gallic acid, caffeic acid, coumaric acid, rutin and cinnamic acid were found in fruit extracts of Alan variety from Erbil province.

Antioxidant Activity

As shown in Fig. 2, the antioxidant activity of the grape samples was influenced by two main factors, namely the variety and the place of sampling. Thus, Ranya and Rost varieties showed the highest antioxidant activity (79%), while Alan variety showed the lowest antioxidant activity in the fruit extract (41%).

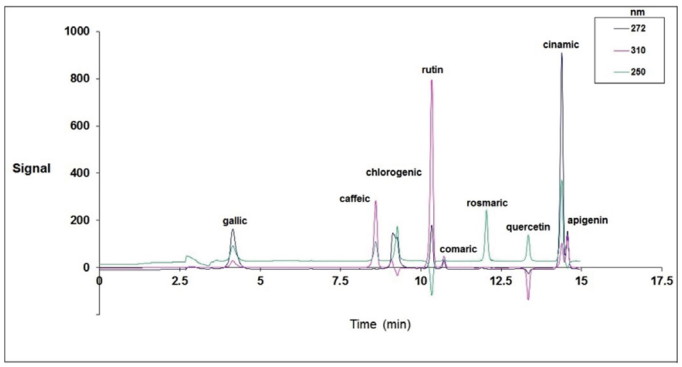

Figure 1. HPLC chromatograms of nine biochemical standards

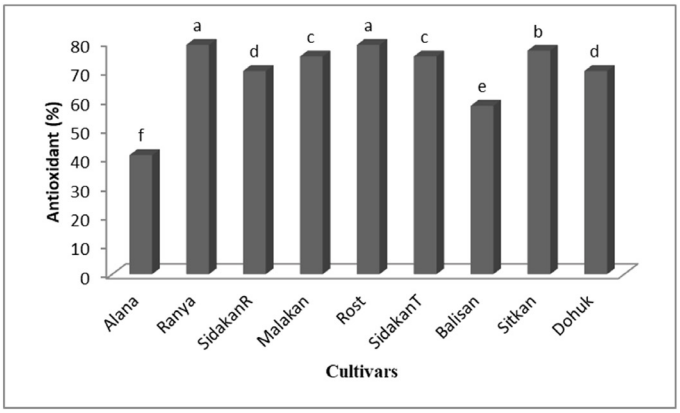

Figure 2. Antioxidant activities of different grape cultivars by DPPH assay

Total Phenolic Content (TPC)

Fig. 3 shows the TPC content of the samples of the grape varieties. The TPC contents in the fruit extracts ranged from 20.3 mg GAE 100 mL^{-1} for the Alan variety to 110.5 mg GAE 100 mL^{-1} for the Sitkan variety. The TPC content of the extracts was significantly influenced by both the genotype and the location of the sample.

Table 2. Comparison of the levels of various biochemical components found in the fruits of different grape cultivars

Cultivars	Gallic acid (mg kg^{-1})	Caffeic acid (mg kg^{-1})	Chlorogenic acid (mg kg^{-1})	Rutin (mg kg^{-1})	Coumaric acid (mg kg^{-1})	Rosmaric acid (mg kg^{-1})	Quercetin (mg kg^{-1})	Cinamic acid (mg kg^{-1})	Apigenin (mg kg^{-1})
Alana	763.62^{a}	19.07^{a}	775.95^{b}	3.58^{a}	107.51^{a}	5.2^{d}	7.66^{f}	2.34^{a}	9.75^{e}
Ranya	608.14^{b}	14.55^{b}	106.97^{g}	0.92^{f}	43.82^{e}	6.81^{b}	19.36^{c}	1.63^{c}	11.87^{c}
SidakanR	559.28^{c}	8.75^{d}	988.36^{a}	2.83^{c}	96.16^{b}	N.D	25.54^{b}	1.5^{d}	8.46^{f}
Malakan	336.08^{d}	11.06^{c}	418.98^{e}	0.4^{i}	30.77^{f}	5.38^{d}	16.15^{e}	0.95^{g}	4.36^{h}
Rost	151.72^{e}	8.39^{e}	665.71^{d}	2.43^{d}	47.47^{d}	1.91^{e}	18.64^{d}	0.38^{h}	1.76^{i}
SidakanT	80.63^{f}	5.54^{f}	103.18^{h}	0.45^{h}	19.68^{g}	0.57^{f}	3.72^{g}	1.0^{f}	22.9^{b}
Balisan	48.25^{h}	2.49^{h}	131.68^{f}	0.84^{g}	16.81^{h}	5.55^{c}	8.41^{e}	2.16^{b}	6.24^{g}
Sitkan	34.74^{i}	4.35^{g}	77.92^{i}	1.42^{e}	13.36^{i}	N.D	52.98^{a}	1.05^{e}	23.04^{a}
Dohuk	54.29^{g}	1.94^{i}	668.86^{c}	3.5^{b}	47.49^{c}	36.32^{a}	8.41^{e}	0.39^{h}	10.57^{d}

Note: The meanings of words that begin with the same letter are not significantly distinct from one another ($P \leq 0.05$). ND: not detected.

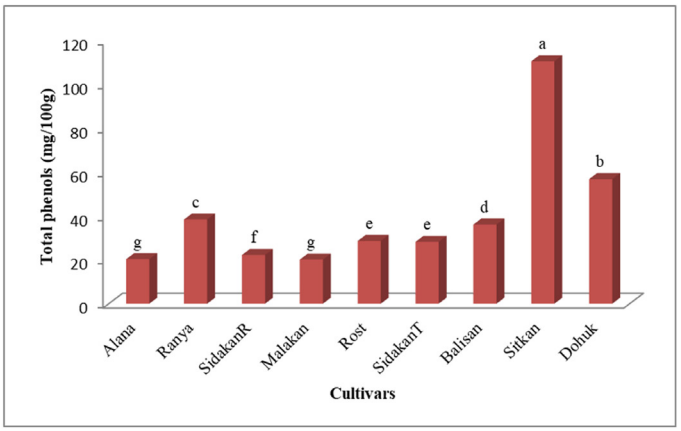

Figure 3. Total phenolic content (TPC) of different grape cultivars

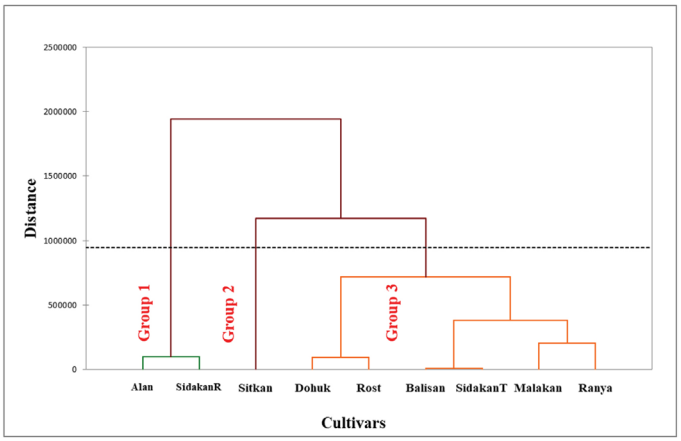

Figure 4. Hierarchical cluster analysis (HCA) of grape cultivars according to the 11 primary characteristics

Classification of Grape Cultivars

HCA and PCA classified the grape variety according to the 11 main characteristics (TPC, DPPH, caffeic acid, gallic acid, chlorogenic acid, cinnamic acid, rosmarinic acid, p-coumaric acid, quercetin, rutin and apigenin). Cluster analysis was performed using the Ward linkage method (Fig. 4). The grape varieties were divided into three main clusters based on this analysis. In the first cluster, Alan and SidakanR were designated as the varieties with the highest values for gallic acid, rutin, caffeic acid, coumaric acid, chlorogenic acid and cinnamic acid. In the second cluster, the variety Sitkan was designated due to the highest levels of quercetin, apigenin and TPC. The third cluster included the varieties Rost, Dohuk, Balisan, SidakanT, Malakan and Ranya due to the highest levels of rosmarinic acid and antioxidant capacity.

The results of the cluster analysis were confirmed by PCA classification (Fig. 5). A PCA was performed to simplify the multidimensional graphs and produce a two-dimensional map explaining the observed variance. The first and second components of PCA explained 62.04% of the total variance (42.20% for PC1 and 19.84% for PC2). The first component (PC1)

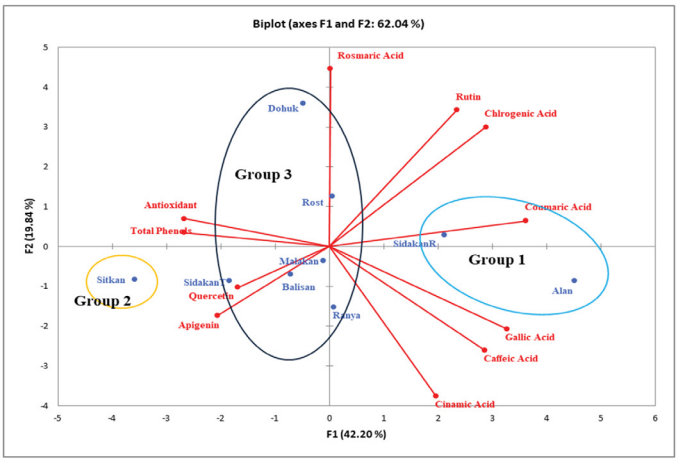

Figure 5. Shows a principal component analysis (PCA) of grape varieties using the 11 primary characteristics

Note: The blue, yellow, and black shapes, respectively, are meant to represent the first, second, and third groups that make up the cluster.

has a strong relationship with gallic acid, caffeine, chlorogenic acid, coumaric acid, apigenin, antioxidant and TPC. The second principal component (PC2) subdivides the samples based on the concentrations of rutin, rosmarinic acid and cinnamic acid.

Discussion

Gallic acid, caffeic acid and quercetin concentrations in grape cultivar fruit extracts were 354 mg kg^{-1}, 15.3 mg kg^{-1}, and 4.1 mg kg^{-1}, respectively. Fruit phenolic composition production can be endogenously controlled during various developmental stages (Naser. et al., 2019), susceptible to external agents. Exogenous factors influencing biosynthesis and accumulation of phenolic compounds in medicinal plants include environmental circumstances (temperature, light intensity, abiotic and biotic stress, humidity) and agricultural techniques (soil fertility, irrigation) (Ghasemzadeh et al., 2012; Alirezalu et al., 2018). The amino acid phenylalanine, which is synthesis during the shikimic acid pathway, functions as a basic structure for a variety of other phenolic compounds. They come into existence as a result of the phenylalanine in question being deaminated by the enzyme phenylalanine-ammonia lyase (PAL) (Shahidi and Chandrasekara, 2010). One of the most important elements in phenolic metabolism is environmental influence, which is, incredibly, light. The synthesis of phenolic compounds is prompted by the action of light, which has an effect on PAL (Macheix et al., 2017).

Wang et al. (2003) stated that the content of phenolic compounds dramatically increased as temperature and carbon dioxide (CO_2) concentration increased. As a result, the differences in phenolic compound genotypes from this study might be attributed to environmental factors such as geographical variances (altitude, height and latitude), temperature, and light intensity.

Bucic´-Kojic et al. (2007) showed the value of antioxidant capacity by DPPH assay in grapes from 45% to 82%, which agrees with our former findings. Assessing the fruit extract antioxidant capacity revealed that this cultivar possessed significant antioxidant potential caused by simple phenolics, phenolic acids, anthocyanins and flavonoids. It was suggested that β-carotene, butylated hydroxytoluene, vitamin C and phenolic compounds had the strong radical-scavenging activity in fruit extracts (Yildiz et al., 2014). Grape extract phenolic content, which includes rutin, chlorogenic acid, caffeic acid and apigenin, is substantially correlated with antioxidant activity. It was revealed that rutin and caffeic acid were markedly associated with antioxidant activity, which is consistent with previous findings (Chen and Ho, 1997; Mariangel et al., 2013). Castelluccio et al. (1995) found that caffeic acid and chlorogenic acid had the highest antioxidant activity compared to p-coumaric acid. Cos et al. (2002) indicated that caffeic acid possessed the plentiful antioxidant activity in fruits.

TPC of grape fruits has previously been observed to range between 99.7 and 121 mg GAE g^{-1} dry weight (DW) among grape cultivar varieties evaluted in Spain (Garcia-Jares et al., 2015). Motalleb et al. (2005) T The total polyphenol content per fresh gram of barberry fruits was, on average, 34.5 mg GAE (FW). Some researchers believe that plant TPC is affected by factors such as longitude, genetic background, sample site, preharvest and postharvest circumstances and maturation stage at harvest.

PCA and HCA were viable methodologies for determining grape genotype classification. Many phytochemical investigations on various grape genotypes and cultivars have been published, demonstrating their antioxidant potential. According to numerous study organizations, anthocyanins, phenolic acids and flavonoids found in grape cultivar fruits have antioxidant properties.

Conclusion

Significant differences were found between the different varieties with regard to the amount of polyphenols present. This fact shows that the relationship between genotype and the environment is one of the most important elements associated with the accumulation and concentration of grape polyphenols. These results show that different grape varieties have the potential to be useful sources of naturally occurring antioxidants and phenolic compounds that could be beneficial to the food industry. In addition, multivariate analysis proved to be a suitable method for classifying the grape samples.

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