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Department of Food Science and Human Nutrition

Evaluation of grape and wine quality parameters of Agiorgitiko (*Vitis vinifera* L. cv.) cultivar grown in Nemea.

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Directed by Dr. Stamatina Kallithraka

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Περίληψη.

Το Αγιωργίτικο (Vitis vinifera) είναι μία ελληνική ερυθρή ποικιλία αμπέλου που καλλιεργείται σχεδόν αποκλειστικά στην περιοχή της Νεμέας, την πιο σημαντική αμπελοπαραγωγική περιοχή της Νοτίου Ελλάδας. Λόγω της σπανιότητας της ποικιλίας και της περιορισμένης της καλλιέργειας, υπάρχει έλλειψη δημοσιευμένων επιστημονικών ερευνών σχετικά με την σύσταση του γλεύκους και των οίνων της ποικιλίας. Σκοπός της παρούσας έρευνας είναι η καταγραφή και αξιολόγηση της χημικής σύστασης σταφυλιών Αγιωργίτικου προερχόμενων από επιλεγμένους αμπελώνες και η σύνδεσή της με την τελική ποιότητα των παραγόμενων αντίστοιχων οίνων. Για τον σκοπό αυτό, αντιπροσωπευτικά δείγματα σταφυλιών συλλέχθηκαν και οινοποιήθηκαν από δεκατέσσερις αμπελώνες από διαφορετικές υποζώνες της περιοχής της Νεμέας, για τρία συναπτά έτη. Εκτενείς χημικές αναλύσεις έγιναν τόσο στα σταφύλια όσο και στους παραγόμενους οίνους και στην συνέχεια οι οίνοι εξετάστηκαν οργανοληπτικά από έμπειρο πάνελ γευσιγνωστών.

Αρχικά στα δείγματα σταφυλιών έγιναν όλες οι κλασικές αναλύσεις (σακχαροπεριεκτικότητα, οξύτητα κτλ) και καταγράφηκαν τα χαρακτηριστικά των ραγών (μέγεθος, βάρος, χρωματισμός γιγάρτων κτλ). Στην συνέχεια ακολούθησε προσδιορισμός των φαινολικών συστατικών τόσο με φασματοφωτομετρικές μεθόδους (DMAC, Folin-Ciocalteau, BSA κτλ) όσο και με ανάλυση με Υγρή Χρωματογραφία Υψηλής Απόδοσης (HPLC). Επίσης πραγματοποιήθηκε μελέτη της χημικής δομής των προανθοκυανιδιών (ΠΑ) μετά από εκχύλιση με οργανικούς διαλύτες και αντίδραση με φλωρογλουκινόλη με HPLC και ανιχνευτή μάζας (MS). Τέλος στο γλεύκος που προέκυψε από την έκθλιψη πραγματοποιήθηκε προσδιορισμός αζώτου με την χρήση

φασματοφωτομέτρου (μέθοδος NOPA) και μεμονωμένων αμινοξέων με τη χρήση Υγρής Χρωματογραφίας Υψηλής Απόδοσης (HPLC). Στους παραγόμενους αντίστοιχους οίνους εκτός των παραπάνω χημικών αναλύσεων πραγματοποιήθηκε επιπλέον οργανοληπτική αξιολόγηση από έμπειρους δοκιμαστές με απώτερο στόχο την αξιολόγηση της τελικής τους ποιότητα.

Η επίδραση των καιρικών συνθηκών κατά την εποχή του τρυγητού ήταν εμφανής στην φαινολική σύσταση των ραγών όπως αυτή εκτιμήθηκε με τις φασματοφωτομετρικές αναλύσεις, Το 2013 παρατηρήθηκε το χαμηλότερο φαινολικό δυναμικό ενώ το 2012 το υψηλότερο. Παράλληλα δεν παρατηρήθηκε επίδραση του βάρους των ραγών στο φαινολικό δυναμικό των σταφυλιών όσο και στην περιεκτικότητα σε ανθοκυάνες (εκφρασμένα ως mg/100 g φρέσκου βάρους) ενώ υπογραμμίστηκε η σημασία της αναλογίας μεταξύ φαινολικών συστατικών φλοιών / γιγάρτων και η επίδραση της στο συνολικό δυναμικό της ποικιλίας.

Ο προσδιορισμός των ανθοκυανών επιβεβαίωσε την μεγάλη περιεκτικότητα της ποικιλίας σε ανθοκυάνες ενώ η ανάλυση των προανθοκυανιδινών η οποία πραγματοποιήθηκε για πρώτη φορά έδωσε πληροφορίες σχετικές με την χημική τους δομή (μέσος βαθμός πολυμερισμού, ποσοστό εστεροποίησης με γαλλικό οξύ και το ποσοστό των προδελφινιδινών) η οποία έχει άμεση συσχέτιση με την αίσθηση του στυφού και την γεύση του πικρού των οίνων. Η επικρατέστερη υποομάδα προανθοκυανιδινών ήταν η (-)-επικατεχίνη και η (-)-επιγαλλοκατεχίνη τόσο στους φλοιούς όσο και στα γίγαρτα των ραγών. Οι προανθοκυανιδίνες των γιγάρτων ήταν σε μεγαλύτερο ποσοστό εστεροποιημένες με γαλλικό οξύ και πολυμερισμένες έναντι των ΠΑ των φλοιών. Αν και η σύσταση των προανθοκυανιδινών των φλοιών ήταν παρόμοια

με την σύσταση των προανθοκυανιδινών των οίνων, δεν αποδείχθηκε ποσοτική σχέση μεταξύ τους. Οι κλιματολογικές συνθήκες φάνηκαν να επηρεάζουν τις ΠΑ των φλοιών ενώ το υψόμετρο τις ΠΑ των γιγάρτων. Η χαμηλές τιμές μέσου βαθμού πολυμερισμού και εστεροποίησης των ΠΑ με γαλλικό οξύ, συνδυαζόμενες με την υψηλή περιεκτικότητα των εκχυλισμάτων σε (-)-επιγαλλοκατεχίνη, σχετίζεται πιθανώς με την χαμηλή αίσθηση στυφού που χαρακτηρίζει την ποικιλία Αγιωργίτικο.

Τέλος, οι αναλύσεις αζώτου και επιμέρους αμινοξέων φανέρωσαν την έλλειψη αζώτου στην συντριπτική πλειοψηφία των δειγμάτων χωρίς να είναι δυνατόν να προσδιοριστεί αν οφείλεται στην ποικιλία ή στις καλλιεργητικές πρακτικές. Κανένα από τα δείγματα δεν περιείχε αμμωνιακό άζωτο σε συγκέντρωση μεγαλύτερη από 2 mg/L. Τα αμινοξέα με την μεγαλύτερη συγκέντρωση σε φθίνουσα σειρά ήταν η αργινίνη, η προλίνη, η γλουταμίνη και το γλουταμικό οξύ. Βάσει του λόγου αργινίνης/προλίνης, η ποικιλία Αγιωργίτικο είναι σύμφωνα με την βιβλιογραφία ασθενής ή ουδέτερος συσσωρευτής αργινίνης. Επίσης παρατηρήθηκε αυξητική τάση της συγκέντρωσης συγκεκριμένων αμινοξέων με την αύξηση της θερμοκρασίας ενώ το αντίστροφο παρατηρήθηκε με την βροχόπτωση. Οι κλιματικές συνθήκες και η ποικιλία είναι οι πιο σημαντικοί παράγοντες που επηρεάζουν την περιεκτικότητα των σταφυλιών σε αμινοξέα.

Σε όλες τις περιπτώσεις διαφοροποιήσεις παρατηρήθηκαν μεταξύ αμπελώνων και όχι μεταξύ των επιμέρους υπο-περιοχών της περιοχής της Νεμέας. Τα αποτελέσματα των αναλύσεων των οίνων στις περισσότερες περιπτώσεις συμφώνησαν με τα αποτελεσμάτων των αναλύσεων των σταφυλιών.

Καθώς η ποιότητα των οίνων είναι αποτέλεσμα πολλών παραμέτρων, σκοπός της έρευνας στην συνέχεια ήταν η ανάπτυξη ενός αξιόπιστου και απλού στην χρήση εργαλείου βασισμένο στην λήψη αποφάσεων με την βοήθεια πολύ-κριτηριακής ανάλυσης ασαφούς λογικής, μέσω της οποίας θα είναι εφικτό, να κατηγοριοποιηθεί αντικειμενικά η ποιότητα των οίνων μέσω επιλεγμένων χαρακτηριστικών των σταφυλιών. Ο λόγος που επιλέχθηκαν τα συστήματα ασαφούς λογικής είναι διότι εφαρμόζονται με επιτυχία στην ομαδοποίηση πολλαπλών δεδομένων τα οποία στην συνέχεια μπορούν να τροφοδοτήσουν συστήματα πολλαπλών επιλογών. Οι παράμετροι σακχαροπεριεκτικότητα, pH, όγκος ράγας, προσβολή από βοτρύτη, χρωματισμός των γιγάρτων, εκχυλισματικότητα ανθοκυανών, οπτική πυκνότητα (OD520) και φαινολικά των φλοιών (dpell) προσδιορίστηκαν κατά τον τρυγητό και τα μεγέθη εισήχθησαν στο εργαλείο πρόγνωσης ποιότητας. Τα σταφύλια οινοποιήθηκαν και οι παραγόμενοι οίνοι εξετάστηκαν οργανοληπτικά από εκπαιδευμένο πάνελ γευσιγνωστών. Στην συνέχεια η σειρά κατάταξης των αμπελώνων σύμφωνα με το `εργαλείο πρόγνωσης` συγκρίθηκε με την σειρά κατάταξης σύμφωνα με την οργανοληπτική αξιολόγηση και τα αποτελέσματα έδειξαν μεγάλο βαθμό συμφωνίας προτείνοντας ότι είναι εφικτή σε μεγάλο βαθμό η πρόγνωση ποιότητας από τους επιμέρους δείκτες που χρησιμοποιήθηκαν στο `μοντέλο`. Αδυναμίες παρατηρήθηκαν αλλά με βελτίωση της μεθόδου και μικρές τροποποιήσεις θα μπορούσε να αυξηθεί περαιτέρω ο βαθμός συμφωνίας.

Λέξεις κλειδιά: Ποιοτική ταξινόμηση οίνων, Ασαφής λογική, Χημική σύσταση ραγών, Αγιωργίτικο, Οργανοληπτική εξέταση οίνου, Μέσος βαθμός πολυμερισμού προανθοκυανιδινών, Βαθμός εστεροποίησης προανθοκυανιδινών με γαλλικό οξύ,

Προανθοκυανιδίνες, Φαινολικές ενώσεις, Ανθοκυάνες Ερυθρός οίνος, Προδελφινιδίνες, Ανάλυση τροφίμων, Αμινοξέα, Άζωτο, Υψόμετρο, Βροχόπτωση

Επιστημονικό πεδίο: Χημεία, Άλλες Γεωργικές επιστήμες.

Abstract.

Agiorgitiko (*Vitis vinifera*) is an indigenous Greek red grape variety, cultivated almost exclusively in Nemea, the most important vine-growing region of Southern Greece. Due to the uniqueness of the grape variety and its limited cultivation, there is lack of published information concerning its grape and wine chemical composition. The aim of this study is to record and evaluate the chemical composition of grapes of selected vineyards of Agiorgitiko and investigate associations between grape analytical parameters and the quality of the produced wine. For this purpose, representative grapes were harvested and vinified from fourteen commercial vineyards from different sub-regions of Nemea for three consecutive years. A number of chemical analyses were performed in both grape samples and produced wines followed by wine sensory evaluation by experienced panel.

Chemical analyses of phenolic content and composition showed that Agiorgitiko is a variety rich in anthocyanins and the importance of seed/skins ratio to final wine composition was highlighted. Proanthocyanidin analysis confirmed that Agiorgitiko is a low astringency variety as indicated by the low mean degree of polymerization (mDp), low percentage of galloylation (% G) and abundance of (-)-epigallocatechin (EGC) units. However, a possible connection between grape and the corresponding wine proanthocyanidin composition was not observed. Amino acid and nitrogen analysis showed that the majority of samples presented a deficiency in yeast assimilable nitrogen required for successful wine fermentation according to the literature. None of the samples had ammonia content more than 2 mg/lt.

In all analyses performed a strong vintage effect was evident verifying that climatic conditions and cultivar are more likely the most important parameters affecting grape and wine chemical composition. Differences were reported only among the values of individual vineyards and not among sub regions, depicting the great variations that exist in the different sub regions of Nemea.

From the analyses performed eight grape parameters were defined by the oenologist experts participating in the experiment, as indicators of wine quality: total soluble solids, pH, berry volume, botrytis infection, grape seed colorization, anthocyanin extractability, optical density (OD 520) and skin phenolics (Dpell). Furthermore, the knowledge of the experts was utilized in order to evaluate the importance of each parameter applying linguistic weights to each individual parameter. A fuzzy logic multi criteria decision making (FMCDM) system was created and these parameters were used as inputs and the result of the system (output) was the score corresponding to wine quality. The produced wines were sensory evaluated by an experienced and trained panel. The ranking of the vineyards, according to the tasting panel, was compared to the ranking made by the tool and the results showed high general agreement between them, suggesting that the latter was able to model expert knowledge successfully. According to the results, the fuzzy logic multi criteria decision making tool could allow the incorporation of grape quality parameters at harvest into a single index providing grape growers and wine producers with a valuable tool for classifying wine quality. The exceptions observed are related to parameters that even though are important for wine quality is unfeasible to include them into the FMCDM system (e.g. characteristic volatile compounds) and/or attributes not adequately

represented in the tasting sheet (e.g. astringency). Our future work aims to modify the FMCDM system optimizing the representation of wine quality of Agiorgitiko and adapt the system to other Greek and international grape varieties (e.g. Xinomavro).

Key words: Wine quality classification, Fuzzy logic, Grape chemical composition, Agiorgitiko, Wine sensory analysis, Mean polymerization degree of proanthocyanidins, Galloylation degree of proanthocyanidins, Proanthocyanidins, Red wine, Phenolic compounds, Anthocyanins, Prodelphinidins, Food analysis, Amino acids, Nitrogen, Altitude, Rainfall.

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Abbreviations.

%AE	Anthocyanin extractability (%)
%DA	Radical scavenging activity
%ION	Degree of ionization of anthocyanins
%G	Percentage of galloylation
%MP	Contribution of seed tannins total phenol content
%P	Percentage of proanthocyanidins
%vol	Alcohol strength
AA	Amino acids
Ala	L-alanine
ANOVA	Analysis of variance
Arg	L-arginine
Asn	L-asparagine
Asp	L-aspartic acid
BSA	Bovine serum albumin
BV	Berry volume
C	(+)-catechin
Cy	3-O-Glucoside of cyanidin
DPPH	2, 2-diphenyl-1-picrylhydrazyl
Dlp	3-O-Glucoside of delphinidin
DMAC	4-Dimethylaminocinnamaldehyde
Dpell	Skin phenolic content
Dpep	Seed phenolic content
EC	(-)-epicatechin
ECG	(-)-epicatechin-3-*O*-gallate
EGC	(-)-epi-gallocatechin

EGCG	(-)-epi-gallocatechin 3-o-gallate
FIS	Fuzzy interface system
FMCDM	Fuzzy logic multi criteria decision making
His	L-histidine
Hyx	L-hydroxyporline
Gln	L-glutamine
Glu	L-glutamic acid
Gly	L-glycine
Leu	L-leucine
Lys	L-lysine
HPLC	High performance liquid chromatography
MCDM	Multi criteria decision making
MCP	Methyl cellulose precipitable tannin assay
mDP	Mean degree of polymerization
MISO	Multiple input single output
Mlv	3-O-Glucoside of malvidin
Mlv acet	Malvidin-3-O-glucose acetate
Mlv coum	Malvidin-3-O-glucose coumarate
OD	Optical density
OPA	O-phthalaldialdehyde
PA	Proanthocyanidins
PDO	Protected determination of origin
Phe	L-phenylalanine
Pn	3-O-Glucoside of peonidin
Pro	L-proline
Pt	3-O-Glucoside of petounidin
SC	Seed colorization

Ser	L-serine
TA	Titratable acidity
Thr	L-threonine
Trp	L-tryptophan
TSS	Total soluble solids
Tyr	L-tyrosine
Val	L-valine
YAN	yeast assimilable nitrogen

Literature review.

1. Introduction.

Time of grape harvest is probably the most important and challenging viticultural decision for grape producers due to the difficulty of assessing grape maturity in the vineyard and predicting wine quality. The definition of ripeness/maturity is dependent on the purpose of grape use. For example, physiological maturity refers to the attainment of final stage of biological function by a plant part of plant as a whole and for grapevine it is when seeds are fully developed. On winemaker`s point of view, maturity is defined as the stage in the berry ripening process when the attributes of the berries at harvest match perfectly the criteria assigned for berry attributes which produce the highest quality of the specified wine style (Illand et al., 2004). Suggesting that winemaker`s pursuit to achieve the highest wine quality for the given conditions, grape maturity is essential. Therefore, within this thesis, we regard the use of the word `maturity` as the optimum grape composition for the production of red wine and in specific Agiorgitiko.

Clearly, there are many factors that contribute to winegrape composition and quality. Some of them are related to vineyard environment (e.g. temperature, altitude) while other parameters and related to viticultural methods and practices (e.g. rootstock, pruning system, control of diseases). Following are discussed key parameters that are used by wine industry as markers of wine quality.

1.1 Analytical markers of grape and wine quality

1.1.1 Sugar content.

It is generally accepted that grape maturity is important to the overall quality of red and/or white wine (Ough and Alley, 1970), which is strongly related to the concentration of most aroma-active compounds (Fang and Qian, 2006), phenolic composition and colour parameters (Perez Magarino and Gonzalez-San Jose, 2006). Significant changes in phenolic composition occur with respect to maturity indicating a greater potential to elaborate high quality red wines (Ferre-Gallego et al., 2012). The two main hexoses contained in grape juice are D-glucose and D-fructose (Figure 1.1) with their concentration in ripe grape juice between 150-250 g/l, but could be higher in overripe or dried grapes. Most other sugars present in grape juice (e.g. D-arabinose, D- xylose, D-galactose) are in minor content and in most cases do not have a chemical importance.

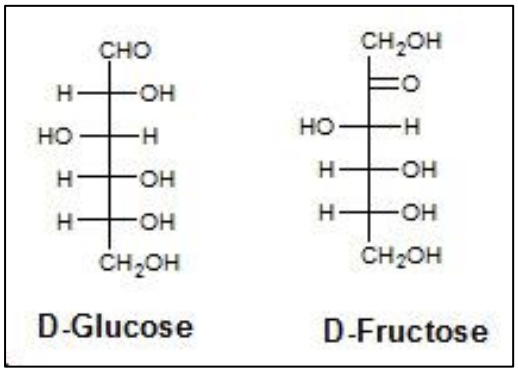


Figure 1.1: Structure of D-glucose and D-fructose.

D-glucose is preferentially fermented by wine yeast and as a result in wines containing few grams of residual sugars, the content of D-fructose is 2-4 times higher than D-glucose. D-fructose is also sweeter than D-glucose, therefore the apparent sweetness of a wine depends on the D-glucose and D-fructose

ratio. Another use of the glucose/fructose ratio is as a marker of maturity, since at veraison, is on the order of 1.5 and drops below 1 at full maturity, mainly due to the action of epimerase, increasing the D-fructose ratio (Ribereau-Gayon et al., 2006). In open, aliphatic form (Figure 1.2), glucose has an aldehyde function on carbon 1, whereas fructose has a ketone function on carbon 2. These two sugars are interchangeable by chemical or enzymatic epimerization via enediol, and are thus function isomers.

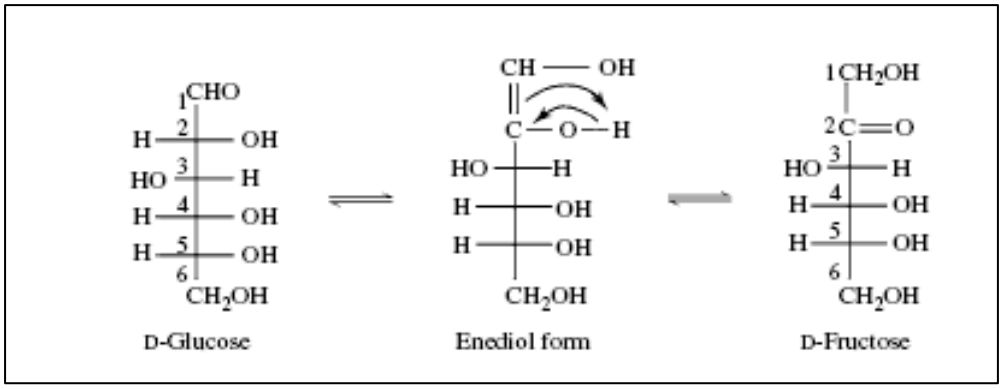


Figure 1.2: Epimerization of D-glucose into D-fructose by enolization.

Grape maturity determined by total soluble solids (TSS) or even by sensory evaluation, is critical parameter (and often the only used) determining the commence of grape harvest. In Greece grape maturity is more often determined by refractometry and / or hydrometry expressed in Baume or Brix indices. Even though many other parameters were proposed (e.g. aroma maturity, acidity), sugar content remains a crucial parameter of grape quality. However, in recent years it is recognised that grape maturity is not adequate to describe grape and wine quality and more precise, reliable and applicable maturity parameters should be found or replace those currently in use (Du Plessis and Van Rooyen, 1982). Indeed, even though the stage of optimum

grape maturity could be determined relatively successfully, it was not possible to predict from these data what the actual level of optimum wine quality would be.

1.1.2 Acidity and pH.

Acidity and pH are very important parameters affecting directly the sensory properties of the wine and regulating the chemical reactions that take place between the wine components. High acidity (low pH) provides advantages in processing and increasing wine quality since among others: increases the antimicrobial and antioxidant properties of sulphur dioxide, encourages the growth of desirable micro-organisms, tends to inhibit wine spoilage, increases the activity of bentonite and enzymes, encourages clarification of must and wine, accentuates the fruitiness and balance of wines, enhances red wine colorization, phenolic stabilization and ageing potential of the wines (Illand et al., 2000; Rankine, 2004). In warm climate regions as Nemea, low acidity (high pH) is often an issue related to reduced quality wines and winemakers have to interfere with acid corrections.

Assessments of acidity were also used to define the optimal time of harvest evaluated as either pH or titratable acidity (TA). Changes in acidity are complex and not necessarily a function of `berry age` and thus a sugar / acidity ratio index, as a general predictive value for wine quality was applied. However, such indices are poor markers of wine quality (Guidetti et al., 2010) and their use is gradually abandoned.

Individual organic acid levels and flocculation reflect berry metabolic activity and could be a useful tool in grape quality assessment. Tartaric acid is the

most prevalent organic acid in grape and wine; it is not very widespread in nature but specific in grapes. Malic acid is more widespread in nature and is the second most abundant grape organic acid. These two organic acids are both synthesized in grape leaves and in the grapes and represent on average 90% of the sum of acids in grapes. During maturation tartaric acid concentration remains relatively constant and decreases mainly due to berry enlargement and other physical modifications (Ribereau-Gayon et al., 2006).

In contrast malic acid is a very active intermediary product of grape metabolism. After veraison and during maturation, its concentration gradually reduces as it is consumed as energy source through respiration producing various secondary metabolites, including some aromatic compounds. The biochemistry related to the accumulation and rapid respiration of L-malic acid in grapes has been studied in detail. In more detail and as seen in Figure 1.3, L-Malic acid accumulates in the berry vacuole before veraison via the collective activities of the phosphoenolpyruvate carboxylase (PEPC) and malate dehydrogenase (MDH) enzymes (Blanke and Lenz, 1989; Diakou et al., 2000; Or et al., 2000). The cytosolic PEPC enzyme, well known for its photosynthetic role in C4- and CAM-plants, catalyzes the (3-carboxylation of phosphoenolpyruvic acid to yield oxaloacetic acid and inorganic phosphate. The resulting oxaloacetic acid is further reduced by the NAD-dependent malate dehydrogenase to produce L-malic acid. Oxaloacetic acid and L-malic acid can enter the TCA cycle to produce citrate as well as other metabolites (Diakou et al., 2000). The b-carboxylation of phosphoenolpyruvic acid plays an important role as an anapleurotic CO_2 fixation step that supplies carbon skeletons for other cellular processes such as osmolarity regulation, pH

regulation and nitrogen assimilation (Diakou et al., 2000). Although a high malic enzyme activity during the accumulation phase of malic acid has been noted, the actual contribution to L-malic acid concentration via the reverse malic enzyme reaction, i.e. pyruvic acid carboxylation, was found to be insignificant (Ruffner et al., 1984; Loulakakis et al., 1996).

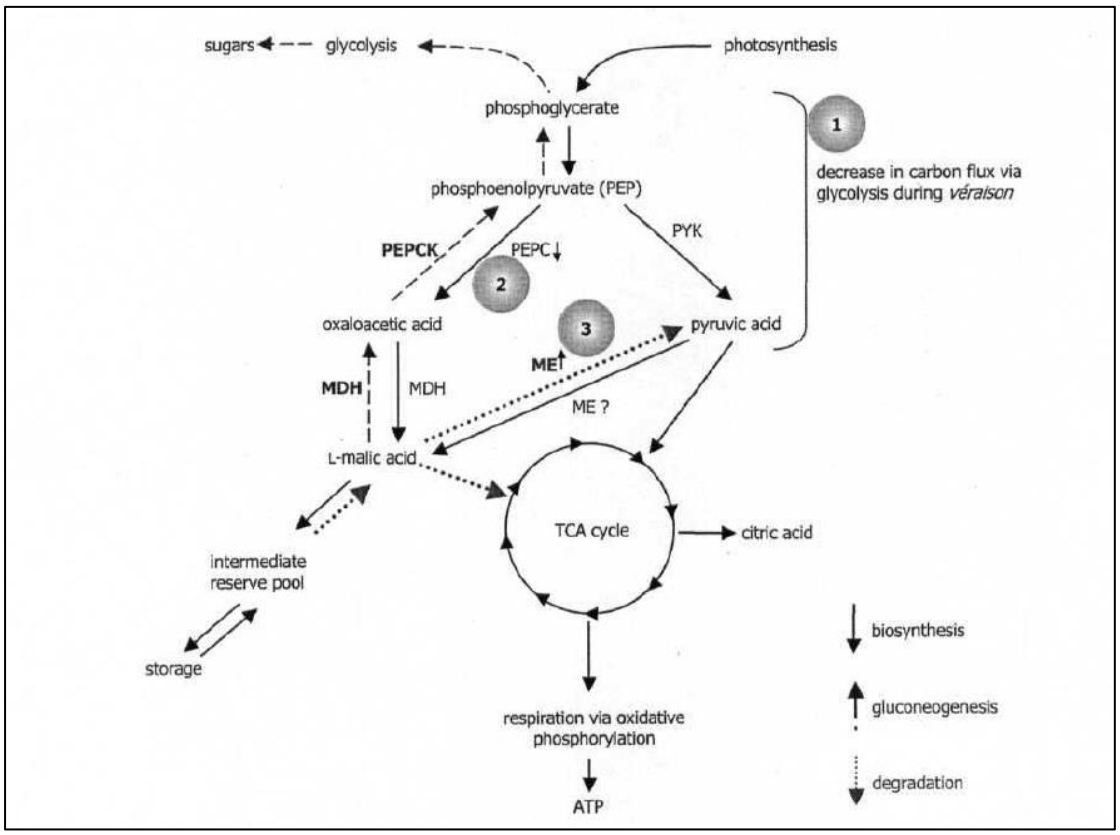


Figure 1.3: The biochemical pathways involved in the biosynthesis, dissipation and regulation of malic acid in grape berries. MDH: malate dehydrogenase, ME: malic enzyme, PEPC: phosphoenolpyruvate carboxylase, PEPCK: phosphoenolpyruvate carboxykinase. The decrease in malic acid is due to a) a decrease in carbon flux via glycolysis, b) a decrease in malic acid biosynthesis via PEPEC and c) an increase in malic acid respiration via the malic enzyme. (Adapted from Kanellis and Roubelakis-Angelakis, 1996).

Therefore, reduced concentration of malic acid in grape juice indicates increased consumption and could be used as an indicator of maturity alone or in combination with tartaric acid content and/or pH/titratable acidity. However, such index is of little true predictive value since the concentration of malic acid is strongly related to the cultivar (Boulton et al., 1996). Furthermore, the

growing / weather conditions, of each vintage affect the rate of respiration, which is significantly slower in cold climates while in warmer climates, tend to be faster (Volschenk et al., 2006).

1.1.3 Berry physical attributes.

Grape berry characteristics (e.g. size, weight, colorization) are important parameters crucial for wine quality and their measurement providing useful information of the expected chemical composition. During berry ripening, changes occur in the physical nature, the chemical composition and the sensory properties of the berry (Illand et al., 2004). Berry ripening is characterized by sugar accumulation, berry weight and volume increase, reduction of acidity, increase in skin colorization, changes in seed colorization and increase in concentration of volatile compounds. Assessing these berry attributes could provide crucial information about berry maturation and though predict wine quality. Even though such methods were introduced early in other food products for the definition and the control of food physical properties (Bourne, 2002); for wine grape berries, the first published attempt to measure and quantify sensory characteristics was made by the Institut Cooperatif du Vin (ICV) in France (Rousseau and Delteil, 2000). Their aim was to design a sensory methodology by which grape-growers and winemakers could assess technological and phenological grape maturity, complemented by traditional maturity measurements (e.g. TSS, pH, TA). Between 1999 and 2003, 2.000 French viticulturists were trained by ICV and used the method to determine harvest date and grape quality. The new methodology, called Quantified Descriptive Sensory Analysis (QDSA) (Rousseau and Delteil, 2000), generated grape berry flavour, aroma and

mouthfeel descriptors based on the international standard ISO 11035 (ISO 1994).

Later the method was adapted by Australian grape-growers and winemakers and was first introduced to QDSA methodology in 2003 through workshops held in several Australian wine regions (Winter et al., 2004). It was promoted as a tool to evaluate grape berry sensory characteristics during the ripening period and to permit assessment of their suitability for a targeted wine style (Winter et al., 2004). The method was called Berry Sensory Analysis (BSA) and since then has been integrated into wine regions throughout the world. The method follows a standardized set of 20 descriptors, assessing the ripeness of wine grapes by judging fruit stems, skin, pulp, and seeds separately (Table 1.1). In more detail, the method evaluates the grape pulp, skin and seeds initially by visual, tactile and consistency assessment; followed by mouthfeel, aroma and taste evaluation. Each attribute is receiving a ranking number according to the established BSA scoresheet and the sum describes the grape maturity, quality and condition.

Table 1.1: Descriptors used for berry assessment by Berry Sensory Assessment (BSA) method.

PULP	SKINS	SEEDS
Visual, tactile and consistency assessment.		
Softness	Colour	Color
Detachment of pulp from skin/seeds	Stalk removal	Crushability
Juiceness	Disintegration	
Mouthfeel, aroma and taste assessment.		
Sweetness	Acidity	Flavors
Acidity	Herbaceaous aromas	Tannic intensity
Herbaceaous aromas	Fruity aromas	Tannic astrigency
Fruity aromas	Tannic intensity	
	Tannic astrigency	

Differences in berry size may affect red wine quality by altering the skin / flesh ratio and modifying the amount of compounds extracted from skins during maceration (Roby et al., 2004; Walker et al., 2005; Matthews and Kriedemann, 2006; Matthews and Nuzzo, 2007). However, Hunter et al. (1991); Johnstone et al. (1995) and Holt et al. (2008), found that relations between berry size, must composition and wine quality were not linear and little is known about its variability in the vineyard and how environmental factors and cultural practices can modify this aspect (Roby and Matthews, 2004). As an example smaller berries could be result of excessive shrivelled berries or water stressed vines. In some other cases when the wine is made of small sized berries, the contribution of seed tannins is excessive, resulting to undesirable coarse mouthfeel. It is therefore suggested that berry size is an important parameter but should be taken under consideration in conjunction with other attributes.

Winemakers often observe changes in seed colorization, taste and crushability since it can assist in estimation of grape ripeness. According to Ristic and Illand (2005), seed maturity is related to the maximum dry weight and minimum extractable tannins of seed. A ripe berry is characterized by skins rich in anthocyanins and complex, relatively inactive tannins and seed with a low content of polymerized tannins that react strongly with proteins. Phenolic maturity explains the quantity and quality of phenols and the capacity to be extracted during vinification (Ribéreau-Gayon et al., 2006). During berry ripening seeds change color from green to yellow, to various stages of brown, to dark brown and black. The changes of seed colorization at different phonological stages are presented in Figure 1.4.

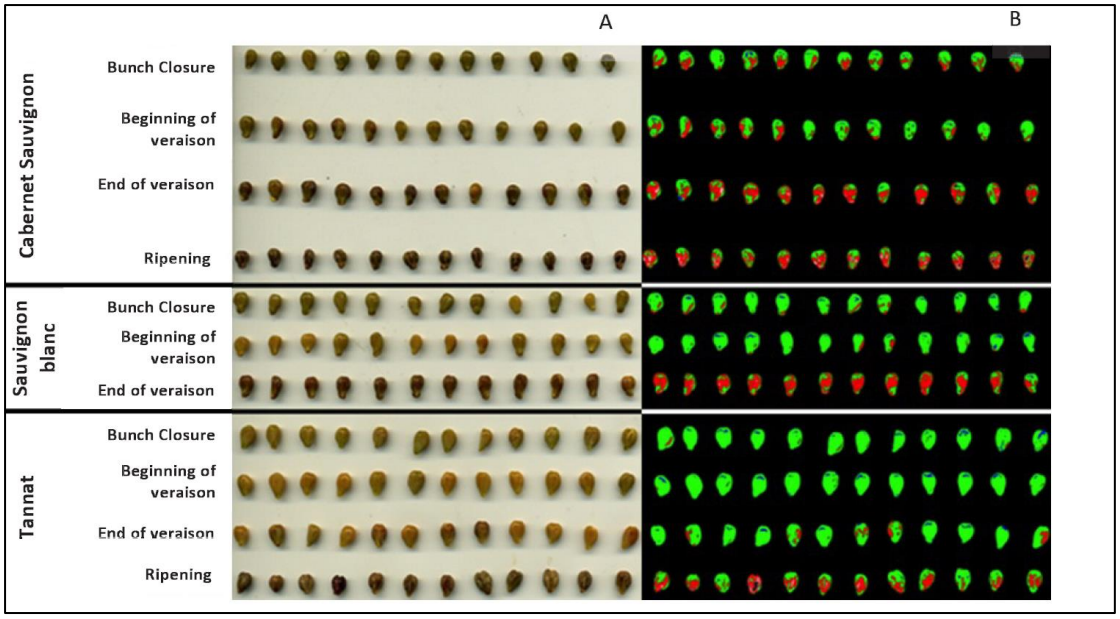


Figure 1.4: Picture of seed from three grape cultivars at four phenological stages. (A) real seeds, (B) after image treatment.

The final mix of colors depends on the variety and the ripening conditions (Fredes et al., 2010; Nogales-Bueno et al., 2017). Since the early work by Ristic and Illand (2005) on the importance of grape seed color as indicator of ripeness, Fredes et al. (2010), developed a comparison method of seed colour against a colour scale (Figure 1.5) while more recently new approaches such as sensory and instrumental texture measurement, FT-NIR analysis or other combined methods for grape seed maturity characterization have been initiated (Torchio et al., 2013; Letaief et al., 2013; Rodríguez-Pulido et al., 2014; Brillante et al., 2015; Rabot, 2017).

Adams and Scholz (2007), estimated that 60% of the wine tannin came from the seeds while only 40% came from the skins. In contrast to skin tannins that frequently are described as “soft” or “ripe,” seed tannins, are associated with more aggressive and less desirable sensory descriptors like “green” or “hard” (Hernandez-Jimenez et al., 2012). Therefore, sensory evaluation and more

importantly bitterness, `greenness` and `dryness` could be indicators of seed tannins quality and content.

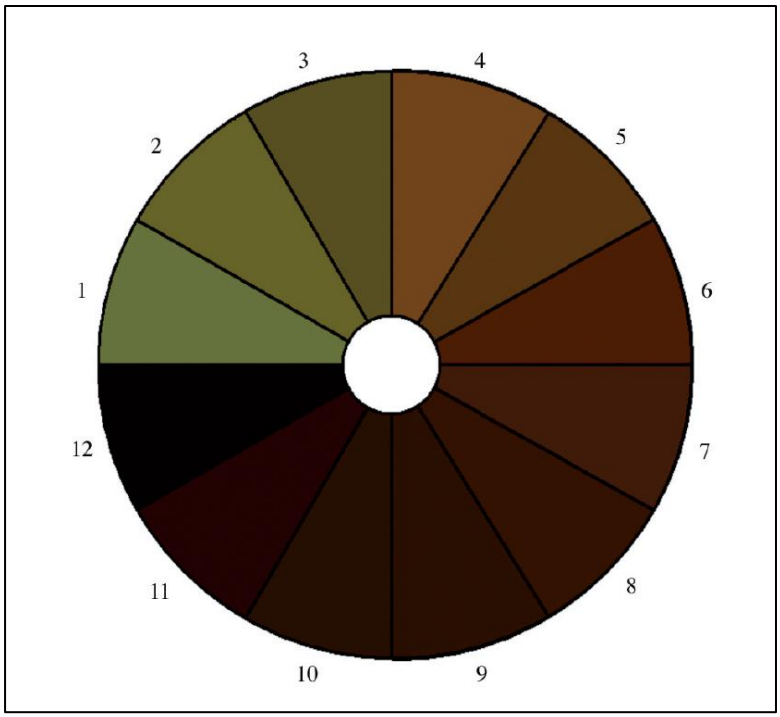


Figure 1.5: Color wheel of grape seed color of *Vitis vinifera* Carmenere.

Sometimes winemakers delay harvest, waiting for the harsher (greener) astringent sensations of seeds to diminish. However, during sensory evaluation seed bitterness may be overwhelming and though distinguishing levels of bitterness / harshness may not be possible. Similarly, seed crushability (hardness) even though is a good indicator of grape maturity (Letaief et al., 2006) it is difficult to define obvious differences especially in the latter stages of fruit ripening.

There is anecdotal evidence reported in the literature suggesting that grape stem ripening may also parallel berry maturation as indicated by a change in stem colour from green to brown during ripening (Ribereau-Gayon et al., 2006; Bisson, 2001; Watson, 2003; Fang et al., 2015). The structure of the

grape stem is presented in Figure 1.6, where the peduncle, rachis, pedicel and lateral grape bunch are indicated.

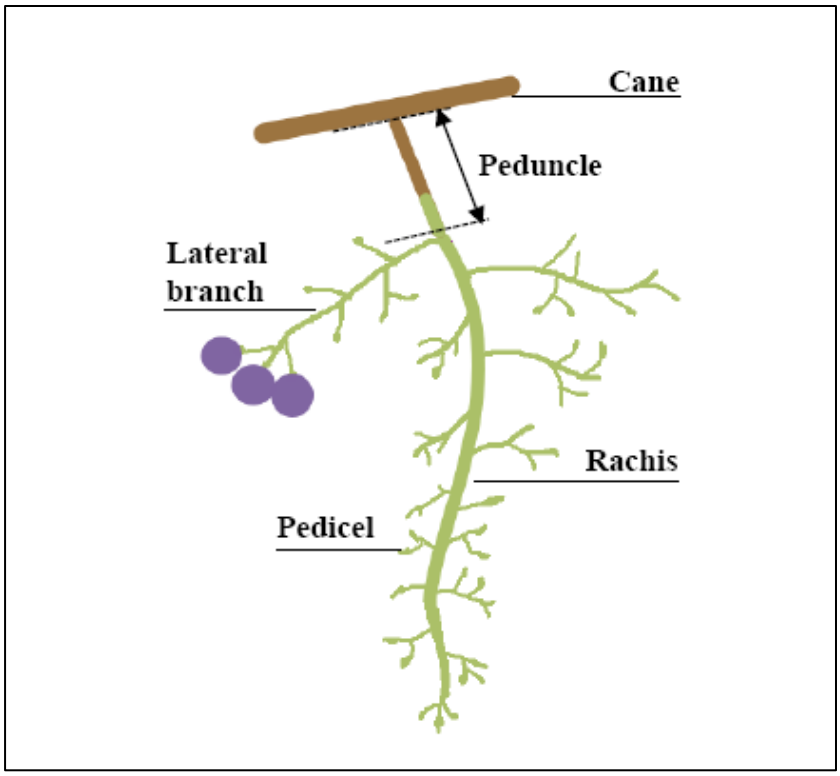


Figure 1.6: Structure of grape stem.

Many winemakers and grape growers assume that there is better tannin maturity (assessed by berry sensory evaluation) with higher peduncle browning (Leal, 2007). In addition to stem colorization, ease of grape detachment from the stem is another parameter that is often used by winemakers to estimate grape maturity. The process of browning of grape shoots is known as lignification and is caused by the death of the green cortex and is accompanied by the deposition of starch in the xylem and phloem parenchyma cells (Plank and Wolkinger, 1976). Fang et al. (2015), confirmed these observations, demonstrating for first time that peduncle moisture content co-develops with the prototypical berry ripeness parameters (TSS, pH, TA). Nonetheless, the stem variability among grape varieties is great as

demonstrated by the OIV descriptor list for grape varieties and *Vitis* species (OIV, 2009), where the lignification content has been used as a visual parameter under the code OIV 206 and 207.

1.1.4 Infection by *Botrytis cinerea*.

Botrytis cinerea is responsible for the one of the most serious grapevine diseases related with quantitative and qualitative deterioration of the grapes (Ky et al., 2012). Considering quantitative aspects, the disease is known to drastically reduce yield at harvest while qualitative; *Botrytis cinerea* is known to affect grape chemical composition and, in particular, to damage the major qualitative compounds such as sugars, organic acids, varietal aromas and phenolic compounds (Ribéreau Gayon et al., 1980). More specifically studies by Peynaud (1984), defined 4 ways in which the grey-mold can negatively affect wine quality: a) Deplete wine color (especially important in red varieties) b) Increase the risk of premature browning (through oxidative enzymes) c) Deplete varietal character (through degradation of grape skins) and d) Contribution to off-flavors developed by the mold ’s presence on the fruit. Preventive measures for *Botrytis* infection include canopy management and other viticultural practices enhancing light and aeration of the canopy, preventing berry damage from natural causes (i.e. birds, hail) or other diseases (i.e. powdery mildew) and spraying with fungicides or copper solutions.

1.1.5 Volatile compounds.

Aroma is an important parameter of grape and wine quality. In their review Robinson et al. (2014), summarised the sources and processes that contribute to wine aroma as follows:

1) The direct contribution of grape-derived aroma compounds, including monoterpenes, norisoprenoids, aliphatics, phenylpropanoids, methoxypyrazines, and volatile sulfur compounds (Ebeler and Thorngate 2009; Gonzalez-Barreiro et al., 2013).

2) Microbially derived secondary metabolites formed from metabolism of sugar, fatty acids, organic nitrogen compounds (pyrimidines, proteins, nucleic acids), and cinnamic acids found in grape (Chatonnet et al., 1992; Herraiz and Ough, 1993; Guitart et al., 1999; Hernández-Orte et al., 2002; Swiegers et al., 2005; Bartowsky and Pretorius, 2009).

3) The contribution of oak-derived aroma compounds that are extracted during fermentation and storage of wine and that vary depending on the origin, seasoning, and heating of the wood (Sefton et al., 1990; Francis et al., 1992; Cadahia et al., 2003; Gómez-Plaza et al., 2004; Garde-Cerdan and Ancin-Azpilicueta, 2006; Fernández de Simón et al., 2010; Garde-Cerdan et al., 2010).

4) Chemical changes associated with acid (Skouroumounis and Sefton, 2000) and enzyme-catalyzed (Günata et al., 1985; Sefton and Williams, 1991; Ugliano, 2009) modification of both non-aroma active and aroma active (e.g., terpenes; Rapp, 1998) grape constituents.

5) Chemical modifications associated with oxidative processes in wine (Simpson, 1978; Escudero et al., 2002; Silva Ferreira et al., 2002), which are related to oxygen uptake from winery operations, storage, and packaging materials (Karbowiak et al., 2009; Ghidossi et al,. 2012).

Several families of compounds are responsible for the aroma of grapes. Among them, esters and terpenes are known to contribute to fruity/floral characters (Capone et al., 2013; Fenoll et al., 2009), C_6-aldehydes and alcohols possess green leafy aroma characters (Kalua and Boss, 2009), methoxypyrazines are strongly linked to green capsicum descriptors (Guillaumie et al., 2013; Genovese et al., 2013). Meanwhile, C_{13}-norisoprenoids generally contribute to many flavors (Peinado et al., 2004), in fruits and wines, such as berry, tobacco, honey, balsamic and violet aromas (Yuan et al., 2016).

Each variety has its own aroma potential which can be influenced by vineyard characteristics and viticultural practices such as soil (Ribéreau Gayon et al., 2006; Gomez-Miguez et al., 2007; Jackson, 2015; Falcao et al., 2008; Coelho et al., 2009), altitude (Allessandrini et al., 2016), weather conditions (Sabon et al., 2002; Jackson et al., 2015; Asproudi et al., 2016; Augustyn and Rapp, 1982; Pons et al., 2017; Crespo et al., 2018), sunlight (Belancic et al., 1997; Bureau et al., 2000; Ristic et al., 2007), training and canopy management (Reynolds et al., 1996; Chapman et al., 2005; Roberts et al., 2007; Allessandrini et al., 2016), rootstock (Marais and Rapp, 1991), water availability (Koundouras et al., 2006; Ribéreau-Gayon et al., 2006; Qian et al., 2009) nitrogen fertilization (Webster et al., 1993; Chone et al., 2006; Garde-

Cerdan et al., 2015), fungicide application (Gonzalez-Rodriguez et al., 2011; Gonzalez- Alvarez et al., 2012; Pinar et al., 2016).

Although the overall composition of most grape varieties is very similar, there are clear and distinct aroma and flavor differences between most cultivars. In red grapes, maximum varietal volatile compounds content is reached at maturity as established by the sugar / acidity ratio, and remains constant in the following weeks (Salinas et al., 2004; Coelho et al., 2009) while in white varieties, changes in the concentrations of volatile compounds during ripening differ with variety, making it more difficult to determine maturity on the basis of varietal volatiles content (Garcıa-Beneytez et al., 2003).

Few aromatic compounds have been directly linked to specific varietal and are presented in Table 1.2. Terpenes (linalool, geraniol, nerol) were the first aromatic compounds linked to Muscat grape varieties (Ribéreau Gayon et al., 1975), while rotundone was the most recent key aromatic identified linked to the peppery aroma of Syrah by Siebert et al. (2008). As the berry ripens there are changes in the types of aroma characters and in the overall level of aroma intensity. Generally in all varieties, herbaceous aromas precursors predominate early, floral and citrus aroma precursors appear in wines when grapes are harvested somewhat later and molecules with the capability to produce fruity aromas seem to emerge latest during the maturity process (Winter et al., 2004). Winemakers pursuit aromas according to their targeted wine style, which often do not coincide with `maturity` as indicated by other indicators like TSS or acidity. As an example the lessening of bell pepper character and the intensifying of forest fruit aromas could be the main indicator of harvesting Cabernet Sauvignon. In addition, studies have showed

(Fang et al., 2012; Yuan and Qian, 2016) that the accumulation of free and glycosylated aroma compounds and precursors during grape ripening are compound dependent, and it is difficult to determine grape-derived aroma because is often subtle and could result from a blend of different compounds. All the above parameters suggest that despite its importance, due to its complicated origin and multiple parameters implicated, grape aroma is a poor indicator of wine quality and cannot provide safe results.

Table 1.2: Impact odorants contributing to varietal aromas. Adapted from Styger et al. (2011).

Odor characteristic	Impact compounds	Cultivar
Floral	Linalool	Muscat
Citrus, floral	Geraniol	Muscat
Citrus, floral	Nerol	Muscat
Geranium oil	Tetrahydro-4-methyl-2-(2-methyl-1-propenyl)-2,5-*cis*-2*H*-pyran (*cis*-Rose oxide)	Gewurztraminer
Kerosene	1,1,6-trimethyl-1,2-dihydronaphthalene	Riesling
Bell papper	3-isobutyl-2-methoxypyrazines	Sauvignon blanc
Coconut, woody, sweet	3,6-Dimethyl-3a,4,5,7a0tetrahydro-3*H*-1-benzofuran-2-one	Gewurztraminer
Black currant	4-methyl-4-mercaptopentan-2-one	Sauvignon blanc
Grapefruit, citrus peel	3-mercapto-1-hexanol (*R* isomer)	Sauvignon blanc
Passion fruit	3-mercapto-1-hexanol (*S* isomer)	Semillon
Black pepper	Rotundone	Syrah

1.1.6 Nitrogen and amino acid compounds.

The nitrogen content of the grape must directly affects the metabolism and multiplication of yeast and thus plays a major role during winemaking since it is related to the fermentation kinetics (Garde-Cerdan et al., 2014). Nitrogen in grapes musts can be found either as organic (proteins, peptides, amines, nitrates, nucleotides, vitamins and mainly amino acids) or inorganic (ammonium salts) (Burin et al., 2016). The amino acid content of the must has a direct effect on wine quality since firstly the varietal aroma could be partially

attributed to the amino acid composition of the grapes (Hernandez-Orte et al., 2009) and secondly during alcoholic fermentation, amino acids undergo various transformations producing flavor-active metabolites such as volatile fatty acids, higher alcohols and esters (Swiegers et al., 2005; Styger et al., 2011). The most important odor related compounds (higher alcohols and fatty acids) are produced from valine, phenylalanine, leucine and isoleucine while serine, threonine, methionine, cysteine and aspartic acid could also form odor impacting compounds (Lambrechts and Pretorius, 2000; Marchand et al., 2000; Ardo, 2006).

Many parameters affect the amino acid composition and content in grapes with variety recognized as the most important determinant of the type and concentration of grape amino acids. The amino acid composition of the grape must affect the synthesis of volatile compounds and in several studies; the varietal aroma could be partially attributed to the amino acid composition of the grapes. In several previous studies (Bell, 1994; Hernandez-Ortez et al., 1999; Stines et al., 2001; Hilbert et al., 2003; Garde-Cerdan et al., 2009) a positive relation between amino acid content and maturity was reported. Other authors (Solari et al., 1988; Hernandez-Orte et al., 1999; Ortegas-Heras et al., 2014) noted that the total amino acid concentration reached its pick level before harvest and then it remained constant or decreased slightly. Jackson and Lombard (1993), reported that a decline in arginine content signals a deterioration of the fruit and though the end of maturation. Later, Garde-Cerdan et al. (2009), confirmed the results and suggested that the reduction of arginine could either indicate the remobilization of nitrogen toward storage organs (i.e. roots) or alternatively arginine could be converted to proline. In all

presented literature, variety is recognized as the most important determinant of the type and concentration of grape amino acids with most other factors related to the environmental conditions and viticultural practices.

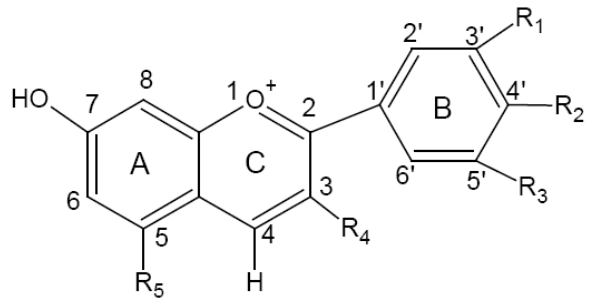
1.1.7 Phenolic compounds.

As discussed, many analytical markers of grape and wine quality were proposed as indicators of wine quality. Concerning red wine quality, with no doubt the group of phenolic compounds is of great interest due to their sensory attributes and chemical properties. However, due to their complicated chemical properties and numerous methods of analysis, few wineries are able to measure and interpretate the analysis results.

Grape phenolic compounds are very important constituents of red wines because, in addition to their antioxidant properties, they contribute to color, astringency and bitterness (Robichaud and Noble, 1990), oxidation reactions (Cheynier and Ricardo da Silva, 1991), interactions with proteins (Ricardo da Silva et al., 1991) and ageing behavior of wines (Haslam, 1980). The most basic phenolic compound is a benzene ring with a single hydroxyl functional group. This simple form does not exist in either grapes or wine, but only considerably more complicated forms, separated into two groups: flavonoid and non-flavonoid. Non-flavonoid compounds are mainly found in grape pulp but are not strictly grape derided since they can be sourced from ageing wines in barrels, winemaking additions etc. The two most well-known classes are the hydroxycinnamates (e.g. coumaric acid, ferulic acid, gallic acid and its derivatives) and stilbenes (e.g. reversatrol). Flavonoid compounds are of

greatest importance for wine quality than non-flavonoid and are divided into anthocyanins, flavonols and tannins.

Anthocyanins are almost exclusively found in grape skins and only few grape varieties contain anthocyanins into the grape pulp. They are responsible for the red color of wines and have no flavor or other organoleptic property. Anthocyanins predominantly exist as glucosides, which form through the conjugation of the flavonoid component, called anthocyanin with glucose. Their structure, flavylium cation, includes two benzene rings bonded by an unsaturated cationic oxygenated heterocycle, derived from the 2-phenyl-benzopyrylium nucleus (Ribereau-Gyon et al., 2006). The common anthocyanins found in grapes are cyanidin, delphinidin, peonidin, petunidin, and malvidin (Figure 1.7), with the latter being the most abundant.



Anthocyanins	R_1	R_2	R_3	R_4	R_5
Pelargonidin 3-*O*-glucoside	H	OH	H	Glucoside	OH
Cyanidin 3-*O*-glucoside	OH	OH	H	Glucoside	OH
Delphinidin 3-*O*-glucoside	OH	OH	OH	Glucoside	OH
Peonidin 3-*O*-glucoside	OCH_3	OH	H	Glucoside	OH
Malvidin 3-*O*-glucoside	OCH_3	OH	OCH_3	Glucoside	OH
Malvidin 3,5-*O*-diglucoside	OCH_3	OH	OCH_3	Glucoside	Glucoside
Cyanidin 3-*O*-rutinoside	OH	OH	H	Rutinoside	OH

Figure 1.7: General structure of wine anthocyanins. Adapted by Han and Xu, (2015).

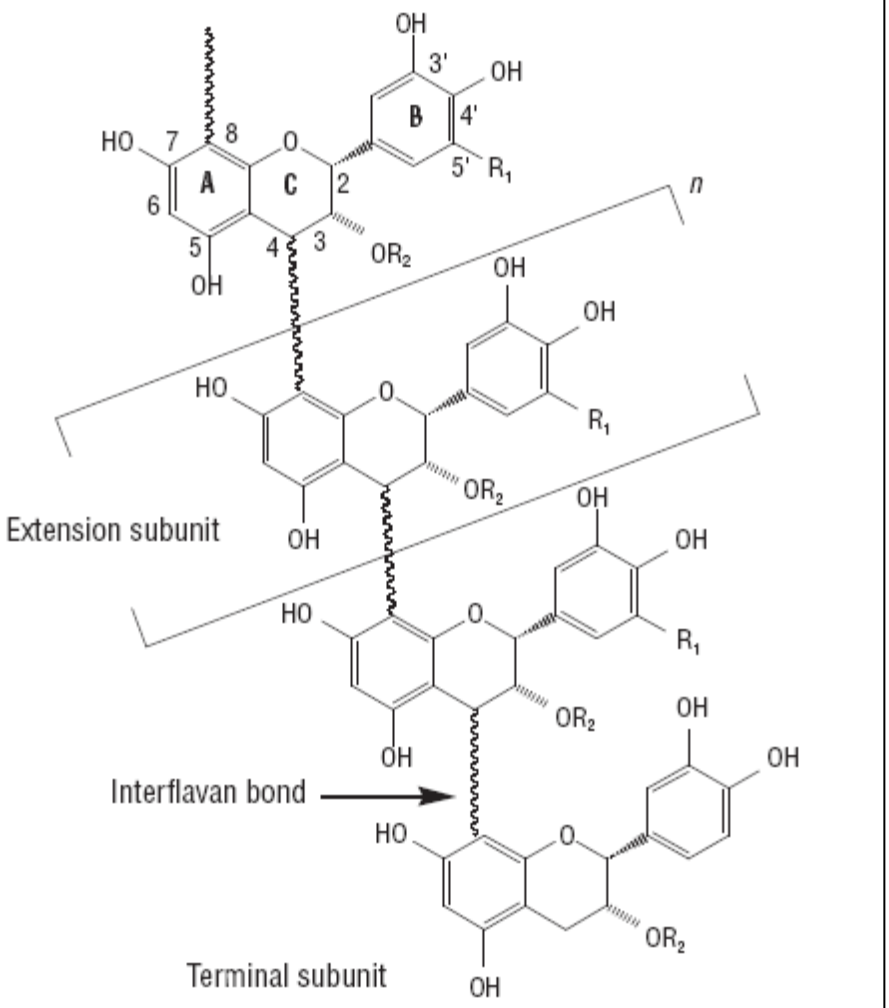
Flavonols are sourced from the grape skin surface and their function is believed to absorb ultraviolet radiation and though provide some protection to

the destructive UV light exposure (Jackson, 2015). They have not yet been attributed a sensory component in wine apart from participating to polymerization with other phenolic compounds. The main flavonols found in *Vitis vinifera* red grape cultivars include the 3-glucosides and 3-glucuronides of myricetin and quercetin and the 3-glucosides of kaempferol and isorhamnetin.

`Tannins` is the common name given to several classes of phenolic compounds and can be divided into two sub categories: hydrolysable and condensed. The class of hydrolysable tannins, so called because the compounds are attached to sugar molecules and which can be cleaved, or hydrolyzed, into their subcomponents, gallotannins and ellagitannins. These are relatively soft tannins found in low concentrations in grape must but their concentration elevates after ageing in wooden vessels, where are found abundantly.

Condensed tannins or Proanthocyanidins (PAs) (Figure 1.8) are important polyphenolic constituents of red grapes. They are found in grape skin and seeds, but their content and properties differ according to the location of the tissues (Curko et al., 2014). Grape tannins derived from skins and seeds vary in their length, subunit composition and sensory properties. Seed PAs are composed of (+)-catechin (C), (-)-epicatechin (EC) and (-)-epicatechin-3-O-gallate subunits (ECG), while skin PAs are composed of (+)-gallocatechin, (-)-epigallocatechin (EGC) and (-)-epigallocatechin 3-O-gallate (EGCg) (Prieur et al., 1994; Escribano-Bailon et al., 1995; Quijada-Morin et al., 2012; Li et al., 2014). Moreover, seed tannins are shorter, with a lower mean polymerization

degree (mDP) and a higher proportion of galloylated subunits (%G) (Souquet et al., 1996; Vidal et al., 2003).



Flavan-3-ol	2-3 Stereochemistry	R_1	R_2
Catechin	trans	H	H
Epicatechin	cis	H	H
Epicatechin-gallate	cis	H	Gallic acid
Epigallocatechin	cis	OH	H

Figure 1.8: Structure of a generalized proanthocyanidin polymer showing a terminal flavan-3-ol subunit and extension subunits derived from flavan-3,4-diols with the inter-flavan bond linking polymeric subunits. The numbering of carbons of the flavan skeleton and flavan-3-ol nomenclature is also shown. Adapted by Downey et al., (2003).

Since tannin isolation and analysis are rather difficult procedures, depolymerisation is often employed to facilitate their characterisation.

Depolymerisation which may be achieved either by thiolysis (Prieur et al., 1994; Monagas et al., 2003) or phloroglucinolysis (Kennedy et al., 2000; Quijada-Morin et al., 2012) allows the subunit profile analysis by high performance liquid chromatography (HPLC). Since the two methods employed are based on different mechanisms for tannin fragmentation by making use of different chemical agents, it is might be possible that the results obtained would be different.

The importance of tannins in the sensory properties of red wine is well documented, particularly with respect to astringency and bitterness. Astringency is a tactile sensation described as drying, roughing or puckering mouth feel that results from the interaction of PAs with salivary proteins (McRae et al., 2010).

According to previous works, the intensity of astringency is directly related with the grape total proanthocyanidin content and their mDP (Chira et al., 2012; Quijada-Morin et al., 2012; Sun et al., 2013); however, this relationship was not confirmed in all studies (Wollman and Hoffman, 2013; Kyraleou et al., 2016). It is also known that subunit composition of PAs is important, with (-)-epicatechin (EC), being more astringent than (+)-catechin (C) (Quijada-Morin et al., 2012; Ferrer-Gallego et al., 2014). Moreover, %G of skin tannins was positively correlated with astringency perception while in seeds the opposite was observed. The presence of EGC in grape skins has been shown to lower astringency perception (Chira et al., 2012).

While the compositional characterization of grape and wine PAs of most international grape varieties such as Merlot, Cabernet Sauvignon, Syrah is

well documented (Mattivi et al., 2009; Chira et al., 2011; Lorain et al., 2011; Quijada-Morin et al., 2012) the information concerning the less known varieties (such as Aglianico, Flavac Mali, Babic, Tempranillo) is rather limited (Curko et al., 2014; Quijada-Morin et al., 2014; Rinaldi et al., 2014).

Anthocyanins, flavonols and tannins accumulate during ripening, influencing the color, taste and mouth feel of wines, though there is evidence to suggest that anthocyanin and flavonol concentrations may decline late in berry development (Bindon et al., 2013; Bindon et al., 2014; Kennedy, 2008; Kennedy et al., 2002).

The highest flavonol concentrations in grapes were found at flowering, followed by a decrease as the grapes increased in size. Subsequently, a significant level of flavonol biosynthesis was observed during berry development and the greatest increase in flavonols per berry can be observed 3-4 weeks post veraison (Downey et al., 2003).

Early studies by Ribereau-Gayon (1972) and Marteau and Schaeffer (1978), indicated that anthocyanins reach their maximum concentration at maturity, when the sugar concentrations attain the highest values. However, the amounts vary greatly according to variety, ecological conditions and viticultural practices (Adams, 2006; Downey et al., 2006; Kennedy et al., 2006).

Biosynthesis of PAs commences after anthesis, reaching a maximum at veraison (Ollé et al., 2011) and subsequently declines until maturity (Downy et al., 2003). Parameters that influence skin and seed PA composition and content are among others: grape variety (Chira et al., 2009; Chira et al., 2011),

grape maturity (Chacon et al., 2009), vine water status (Kyraleou et al., 2016), climatic conditions, vinification practices (Cheynier et al., 1989; Busse-Valverde et al., 2010), botrytis infection (Ky et al., 2012), training system (Kyraleou et al., 2015).

1.1.8 Climatic conditions.

Out of all cultivated plants, the grapevine is considered one of the most responsive to its surrounding environment (Becker, 1984). The climate has a principal influence on both the quantity and quality of the resulting wine and most importantly on the phenological stages of the vine plant. There are a number of factors that affect grapevine physiology and its development summarized in Figure 1.9. Soil, climate, and vineyard practices have direct effects on vine physiology and indirect to vigor stimulation. Changes in vine vigor due to soil, climatic, or management factors can cause changes to canopy microclimate by affecting foliage amount and arrangement in space. Of the cultural practices listed, the training system is singled out for the special role it plays in affecting vine microclimate. Irrigation is an important parameter especially on warm/dry climate since it stimulates shoot, leaf, and fruit growth. In contrast excessive water supply could cause quality deterioration increasing yield and fruit shading.

Out of these factors temperature is the most important parameter since it influences many different biochemical processes important for wine quality (Jackson and Lombard, 1993; de Orduña, 2010; Bonada and Sandras, 2015). The extent of research on the effect of temperature on grapevine is so great that cultivars are often classified in terms of their thermal requirements, and

the prevalent thermal regime is critical to characterize both wines and wine-producing regions worldwide (Jones et al., 2005; Hall and Jones 2009; Keller 2010; Illand et al., 2011). Using the key words ‘temperature’ and ‘grapevine’, the Web of Science (Thomson, 2013) returned 2753 papers showing the increasing interest in temperature effects in viticulture during the last decades (de Orduña, 2010).

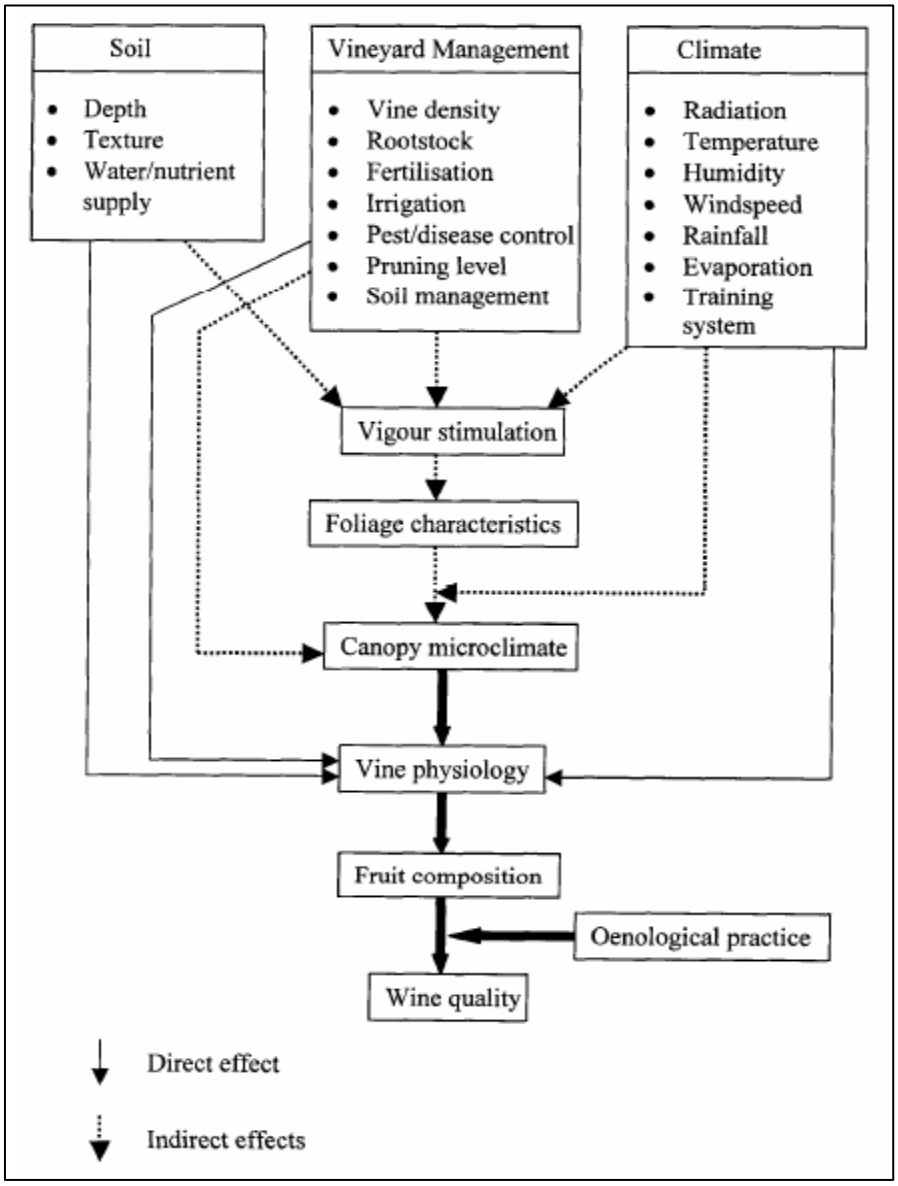


Figure 1.9: Conceptual model to show how soil, climate and vineyard management can affect fruit composition directly or indirectly through canopy microclimate and vine physiology (Adapted by Smart et al., 1985).

Among the grape chemical compounds, temperature influences the production and modification of aromatic compounds (Sadras et al., 2013; Robinson et al., 2014), TSS and acidity (Jackson and Lombard, 1993), phenolic compounds (Downey et al., 2006; Cohen and Kennedy, 2010; Torres et al., 2017), berry characteristics (Sadras and Moran, 2012; Bonada and Sandras, 2015), amino acids (Ortegas-Heras et al., 2014; Bouzas-Cid et al., 2015; Torres et al., 2017).

Irrigation and vine water supply is another important parameter affecting wine quality. Excessive water supply could lead to increased grape production and thus quality deterioration while reduced water supply could influence plant physiology and reduce grape quality. There are studies suggesting that water stress could be beneficial for grape quality mainly on the phenolic composition (Koundouras et al., 2006; Olle et al., 2011) but only under controlled conditions and specific time intervals. Numerous studies are performed on the influence of irrigation on grape and wine quality and even though the results are often contradictory all highlight its importance.

1.2 Assessing grape quality.

As previously discussed, individual grape parameters failed to estimate efficiently wine quality and more complex descriptions are required (Cozzolino et al. 2006; Dambergs et al., 2006; Guidetti et al., 2010). Preferably a method predicting wine quality should include multiple grape parameters, covering multiple layers describing wine quality presenting objective and reliable results. A method to combine multiple parameters, in this case grape characteristics, to rank grape quality for wine production is the Multi Criteria Decision Making

methodology (MCDM). It constitutes a decision support approach that was developed to synthesize an amount of information for the reception of a decision, constantly and logically (Chai et al., 2013; Gal et al., 2013; Yu, 2013). MCDM methods have seen significant use in agricultural and environmental issues. Kiker et al. (2005), provided recommendation for applying MCDM techniques in environmental projects, Okeola and Sule (2012), used MCDM to study urban water supply systems in Nigeria; Jaber and Mohsen (2001), developed a MCDM system for the evaluation of non-convectional water resources supply in Jordan, Kabir (2013), presented MCDM methods for infrastructure management, Baourakis et al. (1996), presented a methodology which combines multi-criteria preference and data analysis in order to design new agricultural products and Krassadaki and Siskos (2000), proposed a MCDM technique to evaluate proposals for rural development projects.

There are several MCDM methods such as (a) Multi-Attribute Utility Theory, (b) Analytic Hierarchy Process (AHP), (c) Fuzzy Set Theory, (d) Goal Programming, (e) ELECTRE, (f) PROMETHEE etc. (Velasquez and Hester, 2013). Between the MCDM approaches available, fuzzy set theory is an efficient tool to model and deal with the imprecise and non-linear nature of practical decision making and classification problems. The major advantage of fuzzy logic based systems over traditional techniques, is their efficiency in handling complex and non-linear problems due to their inherent non-linear character, their capability of adaptation and integration of expert knowledge. Human beings are involved in the decision analysis since decision making should take into account human subjectivity, rather than employing only

objective probability measures. They can be used either in addition to other approaches or as self-reliant methodologies providing thereby a plethora of alternative schemes to work out. In contrast to other approaches that are mostly quantitative approaches, fuzzy logic addresses the problem of data classification in a rather unified qualitative and quantitative manner (Raptis et al., 2000).

The use of fuzzy logic functions has the advantage of reaching solution based on linguistic fuzzy rules and variables which have clear physical meanings. Therefore, fuzzy logic in agriculture would provide more clear results when using data sets from agricultural systems that are very variable and dependent on numerous environmental (soil and water resources, meteorological data) and agronomic (soil tillage, irrigation, fertilization, etc.) parameters. A variety of fuzzy algorithms for data classification in agricultural systems has been proposed. Morlat et al. (2001), proposed an algorithm to estimate the vigour potential conferred by soil based on soil depth and the degree of weathering of the parent rock and Coulon et al. (2010), completed this algorithm by using a fuzzy expert system. Kaufmann et al. (2009), proposed and developed a fuzzy logic expert system to evaluate the potential plant productivity of restored soils based on measured physical soil parameters. De Gruijter et al. (2011), used fuzzy logic for digital soil assessment, resulting in maps that are broadly similar with the ones produced with Boolean models, but more informational as they indicate areas representing a transition between two original Boolean classes. Kolhe et al. (2011), worked on an intelligent multimedia interface based on a novel approach of rule promotion with fuzzy logic for drawing intelligent inferences

for crop disease management, providing highly-effective interactive user interface on web for live interactions and giving solutions of plant pathological problems in short spell. Chang and Sie (2012), have developed a multi-staged fuzzy logic scheme to calculate the growth rate of crops using environmental factors such as light, temperature and water availability. Papadopoulos et al. (2011), used fuzzy logic to design a decision support system for site-specific nitrogen fertilization based on characteristics of the soil, weather and farming practices. Ashraf et al. (2014), used type-II fuzzy set to develop a fuzzy decision support system of fertilizers application taking cropping time and soil nutrients in the form of spatial surfaces into consideration. A self-adaptive fuzzy inference system for the evaluation of agricultural land in China was developed by Liu et al. (2013). Murmu and Biswas (2015), reviewed all work done on fuzzy logic systems for crop classification showing their advantage to classify crops without a definitive decision about the land cover class to which each pixel belongs. Zareiforoush et al. (2015), used fuzzy logic to develop a hybrid intelligent approach for the quality of milled rice and to design an automatic control system for grading of milled rice in the processing industry.

There have been also some attempts to use fuzzy logic systems in the viticulture and wine-making sector. Raptis et al. (2000), proposed a fuzzy classifier and a neural network for the classification of wine distillates with regard to two distinct features of the products, the aroma and the taste, while Tagarakis et al. (2013), applied fuzzy clustering techniques to develop a simplified procedure for the delineation of management zones in vineyards using soil electrical conductivity, soil depth, topography, NDVI, yield and grape composition (must sugar content, total acidity). Tagarakis et al. (2014),

designed, developed and validated a fuzzy inference system to model grape quality in vineyards based on selected grape attributes (total soluble solids, titratable acidity, total skin anthocyanins and berry fresh weight). Furthermore, many studies use fuzzy logic in order to handle uncertain data. Grelier et al. (2007), built a set of rules in order to explain the relationship between vintage quality, reduced to sugar content, and other available variables.

As stated above, wine quality is based on grape quality and its estimation based on grape composition is a priority. However, such estimation should rely on simple and rapid physicochemical analysis, covering multiple aspects of wine quality without advanced laboratory equipment. A tool that could be able to objectively evaluate grape composition and correlate it with wine quality could be of practical interest to winemakers and was not previously developed. The benefits from establishment of such tool would be multiple: Specific grape producing zones could be identified and classified with respect to grape quality. These grape quality zones (which may not be the same every year) could further determine the commercial value of the grapes and be used by the winemakers to improve control over the winemaking processes according to grape quality. Today, overall quality evaluation of wine is primarily based on the results of sensory analysis. Chemical analyses are inefficient for quality determination, however if they are performed in combination with sensory analysis they could provide information only on specific quality aspects.

1.3 Assessing wine quality.

Wine quality, according to Amerine and Roessler (1976), is easier to detect than define mainly due to the subjective layer of quality. Depending on the population (e.g. wine consumers, winemakers) the perception of quality is different and rely on many parameters such as sensory evaluation, wine defects, price, origin etc.

Sensory evaluation and analysis is an important process for commercial winemaking from harvest to bottling. It provides valuable information about the grape and juice maturity, fermentation process and all post ferment wine additions, trials, processes etc. Sensory evaluation is defined as the scientific discipline used to evoke, measure, analyze and interpret reactions to stimuli from wine using our five senses through visual, odor and mouth feel sensations examination. Through visual examination of the wine we retrieve indications as to the origin, style, quality and maturity as well as revealing some possible faults. Wine assessment includes the evaluation of clarity, color (depth and hue), viscosity and effervescence. Odor quality refers to the unique characteristics of the sensation, usually denoted in terms of resemblance to some particular object (e.g., roses, apples, truffles), category (e.g., flowers, fruit, vegetables), personal experiences (e.g., barnyard, hayfield, East Indian store), or emotional/esthetic perceptions (e.g., elegant, subtle, refined, complex, perfumed) (Jackson, 2002). Through aroma assessment is reported the aroma intensity and clarity, complexity and presence of faults. The perceptions of taste and mouth feel are derived by specialized receptors primarily located in taste buds on the tongue. As with odor, several attributes

are evaluated, among them intensity, duration, clarity, complexity, body, balance and aftertaste.

Numerous sensory evaluation tests are performed involving a range of techniques, each designed to study particular characteristics of wines, how they are perceived, and how they relate to features such as the wine's chemical nature or varietal, regional, and stylistic origin (Jackson, 2002). They are however falling into the following categories:

- Discrimination or difference tests. They are the most relevant to winery operations. It assesses whether two (or a few) wines can be differentiated based on one or more attributes. Panelists may only be asked which is stronger is some aspect or may be required to indicate whether the samples are similar or not, in which case identical pairs are also included. The principal tests used are the duo-trio, triangle and paired comparison test.

- Intensity or ranking ratings tests. Scaling techniques are more informative since they provide information about the magnitude of the differences in preference. In this case panelists are asked to identify the attribute intensity on a predefined scale. The scale could be numeric, hedonic or any other kind of intensity scaling.

- Descriptive analysis tests. The goal of this technique is to quantitatively describe the sensory attributes of the wine. Panelists are initially exposed to the variations of the product, then trained and standardized as panel and finally they are asked to score the product on the basis of each descriptive attribute on an intensity scale (Valentin et al., 2012). Many forms of

descriptive analysis are created but the most common are the Quantitative descriptive analysis, Spectrum analysis and Free-choice profiling.

- Time intensity analysis tests. One of the deficiencies in the methods mentioned above is the absence of any indication of the temporal dynamics of the sensations assessed. Time-intensity analysis partially offsets this deficiency by assessing changes in the intensity of various attributes over time, notably gustatory sensations (Lawless and Clark, 1992; Dijksterhuis and Piggott, 2001).

Matured grapes correspond to elevated alcohol wines, affecting viscosity of the wine, sweetness and most tactile sensations. Acidity is an important parameter influencing all physicochemical parameters and reaction during winemaking. Furthermore acidity is wine`s `backbone` and its balance with wine characteristics (e.g. alcohol, phenolics) strongly affects the overall wine quality. Phenolic compounds are responsible for colorization of red and rose wines (anthocyanins), bitterness and astringency while in some cases could influence wine aromatic profile (vinyl phenols).

It is recognized for centuries that specific viticultural practices result in better fruit composition and hence better wines. Even though advances in winemaking practices have improved the quality of the produced wine; mainly due to better sanitized conditions and additives, still grape characteristics are essential for wine quality.

As previously discussed, the chemical parameters that influence wine quality are identified and studied. Despite the different quality perceptions, a generally accepted and less subjective quality baseline could be established

by linking sensory and chemical measurements to wine quality (Hopfer et al., 2015). Sorely wine analyses results, provide only estimation of wine quality and do not dictate wine quality. It is not uncommon, mediocre wine chemical analyses to correspond to highly appreciated wines. Therefore, a model describing wine quality based only on chemical analyses would fail unless it is coupled or `calibrated` with sensory evaluation.

1.4 Agiorgitiko grape variety.

1.4.1 Cultivation of Agiorgitiko.

Agiorgitiko is an indigenous Greek red grape variety cultivated almost exclusively in Nemea, a vine-growing region in southern Greece. The grape is a late budding and ripening variety that is prone to produce high yields if not kept in check by winter pruning or green harvesting. Agiorgitiko is also very sensitive to fungal infection from botrytis bunch rot, downy and powdery mildew. It tends to produce small clusters of small, thick-skinned berries (Figure 1.10). The variety is highly virus-infected and depending on the virus, plant material and age of the vine; this can lead to issues with ripeness and yields which can affect the resulting quality of the wine.

Agiorgitiko is traditionally trained at goblet system but the last decades it is well adapted to most other training systems (e.g. Royat, Lyre). Generally it performs better in dense plantations (4000-5000 plants per hectare), fertile and well drained soils. Vines are well adapted to most American rootstock (e.g. SO_4, R110, 41B), they are susceptible to potassium deficiency and is medium tolerant to drought. In Nemea, vegetation growth is variable mainly due to altitude differences among the region with budburst commencing at

mid-March, flowering on mid-May, veraison at the beginning of August and grape harvest on mid-September.

Figure 1.10: Bunch of Agiorgitiko.

Vine`s morphology and other characteristics such as phenology are used to distinguish different species and varieties, a technique also known as ampelography. While DNA profiling is available to identify varieties, it is expensive and the ability to identify at least the most important varieties is an important skill for viticulturists and winemakers. For each variety, are established `databases` describing the morphological and other characteristics used for variety identification. In Table 1.3 are presented the Viticultural characteristics of Agiorgitiko according to CPVO – OCVV (Community Plant Variety Office – Office Communautaire des Varietes Vegetales) protocol for distinctness, uniformity and stability tests (UPOV Code: VITIS, Adopted on 01/04/2009; entered into force on 01/01/2008).

The CPVO database is a plant variety system that categories varieties according to their viticultural characteristics and allows intellectual property rights, valid throughout the European Union, to be granted for plant varieties. It is established by the European Commission legislation and used extensively as a database of cultivar characteristics and variety identification.

Table 1.3: Viticultural characteristics of Agiorgitiko according to CPVO – OCVV (Community Plant Variety Office – Office Communautaire des Varietes Vegetales) protocol for distinctness, uniformity and stability tests.

Viticultural characteristic	Description of the characteristic* (number)	Scale range
Time of budburst	medium (5)	1-9
Young shoot	wide open (4)	1-5
Young shoot	dense (7)	1-9
Young shoot	medium (5)	1-9
Young shoot: erect hairs on tip	sparse (3)	1-9
Young leaf: color of upper side of blade	greeen with red dots (2)	1-6
Young leaf: prostrate hairs between main veins on lower side of blade	dense (7)	1-9
Young leaf: erect hairs on main veins on lower side of blade	medium (5)	1-9
Shoot: attribute (before tying)	semi-drooping (7)	1-9
Shoot: color of dorsal side of internodes	red (3)	1-3
Shoot: color of ventral side of internodes	green and red (2)	1-3
Shoot: color of dorsal side of nodes	red (3)	1-3
Shoot: color of ventral side of nodes	green and red (3)	1-3
Shoot: erect hairs on internodes	absent or very sparse (1)	1-9
Shoot: length of tendrils	medium (5)	1-9
Flower: sexual organs	fully dev. stamens and fully dev. gynocieum (3)	1-4
Mature leaf: size of blade	medium (5)	1-9
Mature leaf: shape of blade	pentagonal (3)	1-5
Mature leaf: blistering of upper side of blade	medium (5)	1-9
Mature leaf: number of lobes	five (3)	1-5
Mature leaf: depth of upper lateral sinuses	medium (5)	1-9
Mature leaf: arrangement of lobes of upper lateral sinuses	slightly overlapped (3)	1-4
Mature leaf: arrangement of lobes of petiole sinus	half open (3)	1-9
Mature leaf: length of teeth	medium (5)	1-7
Mature leaf: ratio length/width of teeth	medium (5)	1-9
Mature leaf: shape of teeth	both sides straight (2)	1-5
Mature leaf: proportion of main veins on upper side of blade with anth. col.	absent or very low (1)	1-9
Mature leaf: prostrate hairs between main veins on lower side of blade	medium (5)	1-9
Mature leaf: erect hairs on main veins on lower side of blade	dense (7)	1-9
Mature leaf: length of petiole compared to length of middle vein	equal (3)	1-5
Time of beginning of berry ripening	medium (7)	1-9
Bunch: size (peduncle excluded)	medium (5)	1-9
Bunch: density	dense (7)	1-9
Bunch length of peduncle of primary bunch	short (3)	1-9
Berry: size	medium (5)	1-9
Berry: shape	globose (2)	1-10
Berry: color of skin (without bloom)	blue black (9)	1-9
Berry: ease of detachment from pedicel	moderate easy (2)	1-3
Berry: thickness of skin	medium (2)	1-3
Berry: anthocyanin coloration of flesh	absent or very weak (1)	1-9
Berry: firmness of flesh	moderate firm (2)	1-3
Berry: particular flavor	other than Muscat, foxy or herbaceous (5)	1-5
Berry: formation of seeds	complete (3)	1-3
Woody shoot: main color	dark brown (3)	1-5

Continued

Viticultural characteristic	Description of the characteristic* (number)	Scale range
Mature leaf: length of teeth	medium (5)	1-7
Mature leaf: ratio length/width of teeth	medium (5)	1-9
Mature leaf: shape of teeth	both sides straight (2)	1-5
Mature leaf: proportion of main veins on upper side of blade with anth. col.	absent or very low (1)	1-9
Mature leaf: prostrate hairs between main veins on lower side of blade	medium (5)	1-9
Mature leaf: erect hairs on main veins on lower side of blade	dense (7)	1-9
Mature leaf: length of petiole compared to length of middle vein	equal (3)	1-5
Time of beginning of berry ripening	medium (7)	1-9
Bunch: size (peduncle excluded)	medium (5)	1-9
Bunch: density	dense (7)	1-9
Bunch length of peduncle of primary bunch	short (3)	1-9
Berry: size	medium (5)	1-9
Berry: shape	globose (2)	1-10
Berry: color of skin (without bloom)	blue black (9)	1-9
Berry: ease of detachment from pedicel	moderate easy (2)	1-3
Berry: thickness of skin	medium (2)	1-3
Berry: anthocyanin coloration of flesh	absent or very weak (1)	1-9
Berry: firmness of flesh	moderate firm (2)	1-3
Berry: particular flavor	other than Muscat, foxy or herbaceous (5)	1-5
Berry: formation of seeds	complete (3)	1-3
Woody shoot: main color	dark brown (3)	1-5

***** Numbers indicate the ranking of the each characteristic according to the scale provided in the next column.

** Scale according to CPVO – OCVV, with increasing intensity (1 being the lowest value) or otherwise specified.

1.4.2 Research focused on Agiorgitiko.

Due to its scarcity and limited cultivation, little research has been conducted on Agiorgitiko. The importance of irrigation, water regime and timing is well recognized by many authors on international grape varieties. The impact of irrigation on Agiorgitiko was firstly recognized by Koundouras et al. (1999). In his study conducted in different vineyards in Nemea and under different irrigation regimes he reported a relation between water stress and grape quality, with water stressed vines producing smaller berries with higher sugar level, anthocyanin and tannin content. Koundouras et al. (2006), confirmed the above findings suggesting that early water deficit during the growth period had beneficial effects on the concentration of anthocyanins, total phenolics in berry skins. In addition they reported that water stressed vines seemed to increase glycoconjugates of the main aromatic components of grapes as a

quantitative increase in levels of bound volatile compounds, confirmed not only by chemical analysis but also after conducting wine tasting trials. Later, Koundouras et al. (2013), investigated the effect of post veraison water stress and reported a significant effect of water regime on the anthocyanin and berry phenolic compounds, especially on seed tannins. More recently, Chorti et al. (2016), in their research presented contradictory results with irrigated Agiorgitiko vines; containing higher seed tannins, reduced anthocyanin extractability and wine total phenolics, while reduced water supply had no significant effect on berry size and skin tannins.

Other authors focused on the consequences of some commonly applied viticultural practices on Agiorgitiko. Petropoulos et al. (2011), investigated the impact of the main training systems applied on Agiorgitiko in Nemea (Guyot vs. double Royat), leaf removal and shoot elongation (vertical shoot length was allowed to exceed 1.3 m against the common 1.0 m practiced in the region). They reported no significant differences among training systems while leaf removal and shoot elongation, practices that facilitate light penetration into the canopy were not beneficial for grape anthocyanins and phenolic compounds. In previous study, Chorti et al. (2016), evaluated the practice of leaf removal in combination with two irrigations regimes (irrigated and non-irrigated vines) and reported in contrast that light penetration was beneficial for grape anthocyanins and phenolic content. The impact of vine nutrition and rootstock was investigated by Assimakopoulou et al., (2010). Selected vineyards were grafted on 41B (*V. vinifera* × *V. berlandieri*), Richter 110 (*V. berlandieri* × *V. rupestris*) and own rotted vines and focused on the vine micro nutrients N, P, K, Ca, Mg, Fe, Mn, Zn, Cu and Bo. Few differences were observed among

treatments, related mainly on Ca, K and Mg content. It is clear from the literature presented that the research performed on the cultivar and viticulture parameters and practices is limited and though difficult to compare with other Greek and international grape varieties. However, the emerging need for higher quality grapes is forcing researchers to focus on the viticulture parameters of the cultivar.

Unlike the impact of viticulture methods and practices, conducted research in Agiorgitiko wine was more extended. Most studies were not focused solely on Agiorgitiko but generally about Greek grape varieties and though not performed in deep investigation. Since Agiorgitiko is a red grape variety, wine research was focused mainly on the anthocyanin and phenolic compounds. The anthocyanin fraction in Greek cultivars was the first studied as early as 1982, when Harvalia and Bena-Tzourou (1982), published results with the wine anthocyanin composition of selected varietal wines. Later, Bena-Tzourou and Tsoutsouras (1992); Lanaridis and Bena-Tzourou (1997); Makris et al., (2003) and Kallithraka et al. (2005), showed total anthocyanin content of the variety to vary from 33 to 606 mg/l. Arnous et al. (2002) and Makris et al. (2003), reported that Agiorgitiko is a variety rich in anthocyanins with higher values than most of the varietals they studied similar to the Syrah and Merlot/Cabernet Sauvignon wine blends. It worth mentioning that the above studies were conducted on commercial wine samples in most cases aged in barrels, suggesting that further modification of the anthocyanin composition and content could have taken place. Furthermore, in these studies only few samples were analyzed, in some cases including blends with other international and/or indigenous grape varieties. Makris et al. (2006), analyzed

young wines produced and stored in identical conditions from Agiorgitiko, Mandilaria, Xinomavro, Merlot, Syrah and Cabernet Sauvignon sourced from different regions of Greece. Agiorgitiko presented higher anthocyanin values (total and individually) to Xinomavro, Mandilaria and Merlot, similar values to Cabernet Sauvignon and only Syrah presented significantly higher anthocyanin content. To our knowledge, the most recent data published about Agiorgitiko wine anthocyanin content are from Petropoulos et al. (2011), reporting total anthocyanins values between 899.2 to 1084.2 mg/l, significantly higher values than the previous studies (Table 1.4).

Table 1.4: Anthocyanin concentration (mg/l) of wines produced under two viticultural practices: training system and leaf removal. Values represent means of triplicate determinations ± standard deviation.

Treatment		Dp	Cy	Pt	Pe	Mv	MvAcet	MvCoum	Total
		(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)
Training system	***Vineyard 2***								
	Guyot	13.1±0.01	*ND	29.1±0.09	24.2±0.13	815.8±1.38	76.5±0.14	95.7±0.13	1056.0±1.87
	Double Royat	14.2±0.10	*ND	30.8±0.01	23.2±0.03	791.5±4.57	75.4±0.32	101.1±0.45	1032.1±4.43
	Vineyard 3								
	Guyot	27.3±0.20	1.0±0.02	47.4±0.19	53.6±0.40	809.2±3.65	52.1±0.23	82.8±0.23	1078.1±4.90
	Double Royat	29.7±0.15	1.6±0.05	50.4±0.27	61.2±0.24	784.5±1.15	47.0±0.04	105.9±0.20	1084.2±3.95
Leaf removal	***Vineyard 1***								
	No leaves removed	35.7±0.17	3.0±0.04	44.7±0.17	60.8±0.16	652.7±1.27	38.4±0.14	62.8±0.18	899.2±0.99
	Leaves removed	34.0±0.27	1.9±0.05	47.6±0.04	53.9±0.17	725.7±1.55	43.3±0.06	86.7±0.30	992.8±0.67

Adapted from Petropoulos et al. (2011).

Kallithraka et al. (2001), analyzed commercial wines of various Greek varieties and employed both instrumental (non-colored phenolic compounds, anthocyanins, mineral ions concentrations) and sensory analysis in an attempt to classify the wines according to geographical origin. Even though they did not show any major differences among the wines despite the different geographical origin, they provided valuable analytical results of the different cultivars. Later Makris et al. (2003), investigated the polyphenolic content of

Greek wines and found Agiorgitiko having the highest content of flavonols than the other varieties / blends studies, similar values only to Syrah. In the same study hydroxycinnamates, flavanols, benzoates and stilbenes were measured but no significant results about Agiorgitiko were presented. Flavonols and Hydroxycinnamates were measured and compared between wines from Agiorgitiko, Xinomavro, Syrah, Merlot and Cabernet Sauvignon by Makris et al. (2006). Later Kallithraka et al. (2011), compared the total phenolic compounds of Agiorgitiko, Xinomavro and Mandilaria reporting that Agiorgitiko had the lowest values as well as the high pH levels, suggesting that these wines were perceived as less astringent. These findings confirmed the earlier study by Koussissi et al. (2003), on discrimination of dry red wines of Greece according to flavor, with Agiorgitiko having more smooth mouthfeel. On the same year, research by Petropoulos et al. (2011), in wines produced by Agiorgitiko, the total flavonols, flavanols, hydroxycinnamates and phenolic acids were measured and the values found were similar to the earlier study by Makris et al. (2003).

Stilbenes and more specifically trans-resveratrol (3, 5, 4-trihydroxystilbene), in wines from Agiorgitiko has also been investigated. Dourtoglou et al. (1999), investigated and compared the content of trans-resveratrol of various commercial Greek wines from indigenous Greek grape varieties and blends, among them Agiorgitiko; and reported relatively low levels. Later Kallithraka et al. (2001b), analyzed wine samples for trans-resveratrol applying an improved determination method in wines produced according to the Greek appellation of origin system. They confirmed the results of Dourtoglou et al. (1999), that Agiorgitiko has low trans-resveratrol content, reporting an average of 0.76

mg/l. However, comparing their values with other international cultivars grown in various regions of the world, results were obscure.

The sensory characteristics and attributes of Agiorgitiko have been investigated initially by Dourtoglou et al., (1994); Kallithaka et al. (2001a) and more in depth by Koussissi et al., (2002) and Koussissi et al. (2003). The latter sensory evaluated commercial wine samples (twenty four and twenty seven wine samples respectively) of international and Greek grape varieties and blends assessing aroma and oral attributes. In both studies performed Agiorgitiko was linked with fruity attributes (fruity/berries aroma, fruity aftertaste, fruity taste), while in the study by Koussissi et al. (2003), all Agiorgitiko wines were differentiated from oral attributes: fruity and sweet tastes and aftertaste and smooth mouthfeel. In the same study, concerning aroma 10 of 13 Agiorgitiko wines were linked to fruity (tree fruits) and fruity (berries), as in the previous study, but also vanilla, floral and caramelized; but for the panel, only floral and caramelized were significant. In tasting notes, Agiorgitiko character is described by fruity aromas and ‘velvet tannins’. Astringency was later investigated by Kallithraka et al. (2011), comparing wines from Xinomavro, Agiorgitiko and Mandilaria. Their tasting panel showed that mandilaria was the most astringent variety whereas Agiorgitiko was the least, characterized by smooth mouthfeel and a lower astringency. More recently, Dourtoglou et al. (2014), analyzed the volatile compounds of various Greek and international cultivars focusing on Agiorgitiko and Moschofilero. In Table 1.5 are presented the results which so far are the latest published data about the volatile composition of the variety.

Table 1.5: Concentrations of volatile compounds in Agiorgitiko (mg/100 g of wine).

Code	Compound	Wines									
	Name	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10
C1	3-Methyl-1-butanol	12,564	7,551	11,872	10,763	12,648	10,270	6,376	8,229	12,152	4,312
C2	Acetyl methyl carbinol	1,639	1,948	1,948	1,948	1,948	1,948	1,061	3,285	1,948	1,808
C3	Ethyl isobutyrate	0,118	0,764	0,103	0,050	0,252	0,252	0,122	0,545	0,252	0,060
C4	Butanediol	4,725	2,596	2,490	6,135	3,468	3,420	3,149	3,626	4,639	3,663
C5	2-Methylpropanoic acid	1,745	0,884	0,729	1,838	1,629	1,012	1,350	1,319	1,829	1,367
C6	Ethyl lactate	4,094	3,267	7,418	4,047	4,179	7,841	5,185	3,466	4,759	3,407
C7	3-Methyl-1-butyl acetate	0,630	0,887	0,931	1,009	0,897	1,289	0,224	0,510	0,675	0,309
C8	2,3,-Butanediol	1,483	6,509	1,938	1,704	2,583	2,965	3,096	1,835	3,005	2,946
C9	3-Methyl thiopropanol	0,199	0,254	0,324	0,221	0,307	0,300	0,245	0,125	0,239	0,180
	3-Octanol	11,450	10,700	11,500	10,700	11,300	11,965	12,300	12,000	12,000	12,300
C10	2-Hydroxy pentanoic acid	0,657	0,228	0,234	0,291	0,354	0,267	0,216	0,157	0,301	0,301
C11	Iso amyl acetate	1,289	2,056	3,034	0,849	0,896	1,488	0,526	0,314	1,306	1,306
C12	2-Phenylethanol	6,389	6,551	5,823	4,098	9,466	5,012	5,724	3,978	5,445	5,210
C13	Diethyl butanedioate	2,589	3,411	1,981	1,736	2,239	2,199	1,191	0,701	1,350	0,609
C14	Monoethyl butanedioate	4,991	4,192	4,351	6,536	16,036	12,376	11,741	9,626	13,383	9,679
C15	Phenil ethyl acetate	0,576	0,566	0,363	0,188	0,350	0,329	0,188	0,109	0,334	0,334
C16	Ethyl-p-hydroxy phenyl propionate	0,274	0,255	0,319	0,238	0,365	0,507	0,335	0,205	0,272	0,308
C17	Di iso amyl butanedioate	2,393	2,025	0,370	1,345	3,604	1,732	2,060	1,293	1,862	1,796
C18	p-Hydroxy phenyl ethanol	1,200	1,760	1,112	0,449	0,708	0,280	0,355	0,205	0,733	0,281
C19	Phenyl ethyl lactate	0,185	0,295	0,384	0,093	0,291	0,650	0,294	0,176	0,296	0,296
C20	Monoamyl butanedioate	0,295	0,379	0,694	0,190	0,580	0,364	0,224	0,389	0,389	0,389
C21	n-Acetyl tyramine	0,459	0,668	0,283	0,210	0,211	0,307	0,208	0,157	0,313	0,313
C22	hexyl butanedioate	0,181	0,172	0,228	0,116	0,233	0,220	0,181	0,181	0,181	0,116
C23	Indole-3-ethanol	0,288	0,359	0,417	0,136	0,365	0,208	0,196	0,240	0,24	0,188
C24	n-Amino acetyl tyramine	0,189	0,189	0,189	0,248	0,189	0,189	0,143	0,187	0,189	0,180
C25	Phenyl ethyl butanedioate	0,288	0,253	0,222	0,442	0,270	0,237	0,220	0,217	0,338	0,180
C26	Ethyl-p-hydroxy cinnamate	0,255	0,284	0,115	0,221	0,169	0,200	0,122	0,152	0,305	0,180
C27	p-Hydroxy cinnamic acid	0,154	0,154	0,156	0,151	0,154	0,154	0,154	0,154	0,154	0,154

Adapted from Dourtoglou et al. (2014).

Few studies focused exist investigating the amino acid composition of Agiorgitiko grapes and wines. Initially, Dourtoglou et al. (1994), stored Agiorgitiko grapes under carbon dioxide simulating carbonic maceration conditions and compared the chemical composition against a control condition. He reported proline and arginine as the AAs in greater quantity followed by serine, alanine and GABA (Gamma aminobutyric acid). The AA composition in red wines was later investigated by Bouloumpasi et al. (2002), analyzing eight Greek and international grape varieties among them nine wine samples of Agiorgitiko. She reported as Agiorgitiko with Kotsifali and Grenache rouge as having higher AA content than the other cultivars examined (Merlot Syrah, Xinomavro, Mandilaria, Cabernet Sauvignon) exhibiting higher concentration of arginine and glutamic acid.

1.4.3 Wine region of Nemea.

The total expanse of Nemea region is 42.951 hectares, out of which only 22.000-25.000 are planted with Agiorgitiko (Figure 1.11). The altitude of the region is between 80 m to 1235 m above sea level but vines are cultivated only in the range between 95 and 850 meters with largest percentage of the vineyards (48.7%) cultivated between 250-500 meters. The mountainous topography of Nemea is characterized by valleys formed by the numerous rivers of the region (Asopos, Xerias, Mavrorema etc).

Figure 1.11: Overview of Nemea Valley.

The central and larger valley is of Nemea (Figure 1.11), including the villages of Galata, Aidonia, Petri and Koutsi, while the other valleys are Ancient Kleones, Ancient Nemea, Leontion-Gymno, Asprokampos-Psari (Figure 1.12), Kefalari and Malandreni.

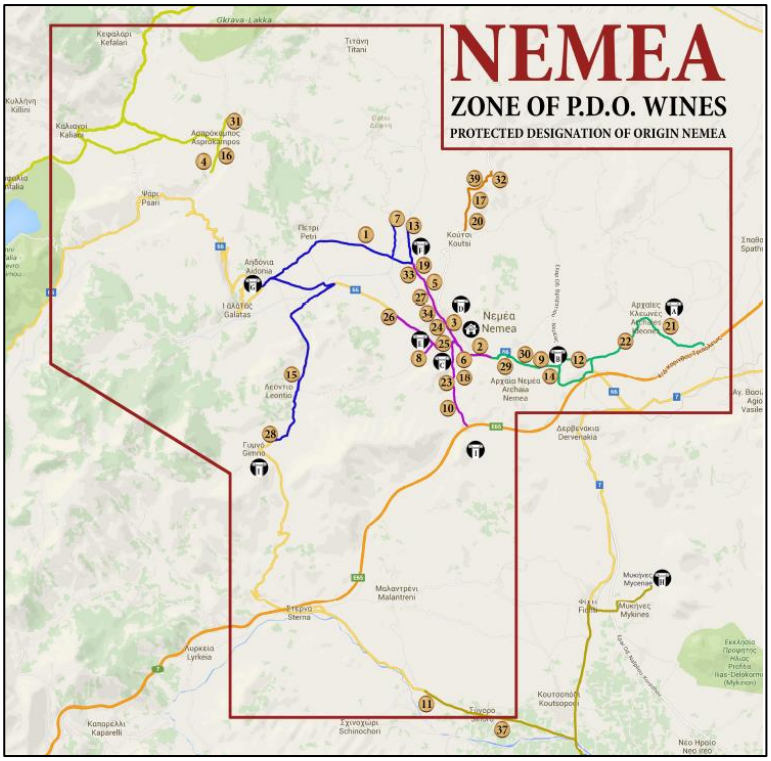


Figure 1.13: Schematic map of the wine region of Nemea.

Concerning climate conditions, Nemea is characterized by Mediterranean climate; mild winter season with adequate rainfall and warm and dry summer season. Winter temperatures are often below 0^{o} C while on summer temperature exceeds 40^{o} C. Annual average temperature is between 16^{o} C to 18^{o} C and annual rainfall is 750 mm. Soil composition is variable among the regions but is generally characterized as low in organic matter, relatively high in calcium carbonate ($CaCO_3$), slightly alkaline (pH 7.0 - 7.5) and poor in nutrients.

Mono-varietal Agiorgitiko is used for the production of 'Nemea', protected designation of origin (PDO) wine; one of the most important appellations of Greek wines. The viticulture area producing `PDO Nemea` wines was initially defined on 1971 by a royal decree and since then has been further modified on 1974, 1988,1996, 2007 and 2009. More specifically the region is oriented by the administrative boundaries of seventeen (17) villages, ten (10) of them

are parts of the municipality of Nemea (Nemea, Aidonia, Ancient Nemea, Ancient Kleones, Galatas, Daphne, Koutsi, Kastraki, Leontion, Petri), five (5) are part of the municipality of Sikyon (Asprokampos, Bozika, Titani, Psari, Kefalari) and two (2) are part of the municipality Argos-Mycenae (Gymno, Maladreni). In addition to the geographical boundaries, for the production of Nemea PDO additional commitments to grape growing, vinification, wine style, aging and wine composition are undertaken and summarized in the European Commission Regulation (2007).

Even though Nemea is the birthplace of Agiorgitiko, its cultivation is expanded and cultivated in most wine regions of Greece. In Table 1.6, are presented the wine growing regions of Greece that Agiorgitiko is authorized or permitted according to European Union Legislation (2919/95506/2017). As seen the cultivation of Agiorgitiko is authorized or recommended in all Greece (with the exception of Greek islands) indicating the importance of this variety for the wine sector, its adaptability and its recognized reputation. Furthermore Agiorgitiko participates in numerous Protected Geographical Indication (PGI) wines of Greece.

Table 1.6: Wine growing regions of Greece that cultivation of Agiorgitiko is authorized, authorized or not permitted (EU 2919/95506/2017).

Wine growing region	Regional units included	
Thrace	Evros, Xanthi, Rodope	Authorized
Macedonia	Grevena, Kozani, Drama, Imathia, Thessaloniki, Kavala, Kastoria, Kilkis, Pella, Pieria, Serres, Chalkidiki, Florina.	Authorized
Epirus	Arta, Thespotia, Ioannina, Preveza.	Authorized
Thessaly	Karditsa, Larissa, Trikala, Magnesia, Sporades.	Authorized
Central Greece	Aitoloakarnania, Attica, Boeotia, Euboa, Evrytania, Fokis, Thiotis.	Recommended
Peloponnese	Argolis, Corinthia, Arkadia, Messenia, Laconia, Ileia, Achaia.	Recommended
Ionian islands	Corfu, Cefalonia, Zakynthos, Lefkada.	Not permitted
North Aegean	Chios, Samos, Lesvos, Lemnos.	Not permitted
Cyclades	Syros, Kea, Milos, Paros, Naxos, Tinos, Myconos, Andros-Thira	Not permitted
Crete	Herakleion, Lasythi, Rethymno, Chania	Authorized
Dodecanase	Rhodes, Kos, Karpathos, Kalymnos.	Not permitted

2. Aims of this study

The lack of objective estimation of enological potential of a vineyard is still the weakest circle in the production of quality wines. Laboratory parameters that have been used and depicted the quality of the grapes and as a result of the produced wine (e.g. sugar content) are not providing safe outcomes of the final product. Specialized analysis that could give us an estimation of the quality of the grapes are time consuming and financially unfeasible, with no practical application. The main goal of our study is the evaluation of Agiorgitiko (*Vitis vinifera* L. cv.) grape and wine quality parameters grown in the wine region of Nemea. The results obtained will be then utilized to evaluate the enological potential of a vineyard, applying simple and low cost physicochemical analysis (e.g. concentration of phenolics, anthocyanin extractability index) in grape samples. This will be feasible through establishing relations between grape chemical composition and wine quality parameters, developing a mathematical model based on the statistical weight of the individual analysis. The potential benefits could be multiple: the wineries, evaluating the quality of the grapes shall guide accordingly the winemaking processes, the grape pricing will correspond to their real enological potential and evaluation of experimental practices (e.g. irrigation) will be facilitated.

More specifically this work was focused on the following specific objectives:

- Assessing the chemical composition of Agiorgitiko grapes in relation to the respective parameters of the international grape varieties, according to bibliography. Investigation was also applied in grape and

wine chemical compounds which are either poorly studied (amino acids) or analyzed for first time (proanthocyanidins) in Agiorgitiko.

- Evaluating Agiorgitiko wine quality employing selected chemical and sensory parameters.
- Developing of a simple in use and reliable tool based on fuzzy logic mutli-criteria decision making to objectively classify wine quality, based on the statistical weight of the individual analysis.
- Investigate the influence of climatic parameters (such as average annual temperature and rainfall) and harvest year on Agiorgitiko grape and wine chemical composition, using meteorological data.

Methods and materials.

3.1 Experimental design.

3.1.1 Introduction.

The study was conducted in the wine region of Nemea, Greece, for three consecutive years (2012-2014). This region was selected as one of the most important wine regions in Greece covering over 3000 ha of vines (Act No 539/4-8-1971). The first two years of the experiment we conducted extended grape and wine analysis in an effort to identify the analyses that could be later used as markers for estimating wine quality. Since Agiorgitiko is a red grape variety, the greatest part of our research was focused on phenolic compounds. There are three main methods to analyze tannins. These are the colorimetric (Schofield et al., 2001), gravimetric (Giner-Chavez et al., 1997) and precipitation methods (Hagerman & Butler, 1978, Harbertson et al., 2003; Sarneckis et al., 2006). To understand tannins and anthocyanins in wine, it is important to investigate the origin of these compounds, their evolution into grape ripening and their extraction into the grape must / wine (Nel, 2018). Part of this study was dedicated to examine the extraction of these tannins into wine and their influence on wine sensory properties. Therefore, these chemical analyses were coupled with sensory analysis of the produced wines providing further information and estimation of wine quality. Combining analytical results, sensory evaluation data, literature and personal communications with wine professionals, we were able to identify a number of grape chemical analyses that could be used as markers of the wine quality. The third year of the experiment, small scale vinifications, sensory evaluation and the selected analyses were performed and a method to combine analytical parameters into ranking wine quality was developed.

3.1.2 Vineyard data.

Fourteen (14) vineyards were randomly selected from five (5) different sub-regions of Nemea as defined by the villages Koutsi, Nemea (valley of Nemea), Tsintaria (west slopes of Koutsi), Ancient Nemea and Asprokampos; in order to have representative samples and from different quality levels as indicated by the viticulturists assisting to our research (see Appendix). The soil of this area is generally characterized as low in organic matter, slightly alkaline (pH 7–7.5) and insufficient in nutrients. Vineyard characteristics are presented in Table 3.1. The altitude of the vineyards was variable (from 270m to 730m above sea level). All vineyards were planted with *Vitis Vinifera* L. cv. Agiorgitiko grafted on American rootstock. Vine age was between 10 and 22 years and planting density between 3780 and 5680 vines per hectare with vine spacing of 2–2.2 m between rows and 1–1.2 m within a row. All viticulture practices (pruning, shoot positioning and harvest) were performed manually. Harvest of grapes was performed according to technological maturity, for production of Nemea PDO wines following the applied legislation (Commission Implementing Regulation, EU/1234/2007).

Table 3.1: Vineyard characteristics.

								Flowering			Veraison			Harvest		
	Sub region	**Altitude (m)**	**Slope**	**Soil Type**	**Orientation***	**Rootstock****	**Vine denisty*****	2012	2013	2014	2012	2013	2014	2012	2013	2014
Vin. 1	Koutsi	490	0 - 5 %	sandy-clay-loam	E - W	41B	3950	4/6	6/6	2/6	23/7	23/7	22/7	12/9	12/9	25/9
Vin. 2	Koutsi	485	0 - 5 %	sandy-loam	E - W	R110	5680	5/6	1/6	2/6	25/7	28/7	27/7	12/9	10/9	12/9
Vin. 3	Tsintaria	390	5 - 10 %	clay-loam	E - W	R110	3950	5/6	1/6	2/6	25/7	28/7	27/7	12/9	10/9	12/9
Vin. 4	Koutsi/tsintaria	410	5 - 10 %	sandy-loam	E - W	R110	3950	5/6	1/6	2/6	25/7	28/7	27/7	12/9	10/9	12/9
Vin. 5	Asprokampos	730	5 - 10 %	sandy -loam	E - W	R110	3950	12/6	10/6	9/6	3/8	5/8	7/8	26/9	17/9	26/9
Vin. 6	Koutsi	550	0	sandy-loam	E - W	R110	3780	1/6	4/6	3/6	23/7	24/7	23/7	14/9	9/9	19/9
Vin. 7	Koutsi	510	5 - 10 %	clay-loam	N - S	41B	3780	4/6	6/6	6/6	28/7	25/7	25/7	14/9	16/9	19/9
Vin. 8	Nemea Valley	275	0	sandy-clay-loam	N - S	R110	3950	1/6	1/6	5/6	5/8	6/8	2/8	22/9	18/9	24/9
Vin. 9	Nemea Valley	270	5 - 10 %	sandy-clay-loam	E - W	41B	3780	3/6	5/6	7/6	2/8	5/8	2/8	22/9	18/9	24/9
Vin. 10	Koutsi	490	0	clay-loam	N - S	R110	3780	6/6	6/6	6/6	25/7	22/7	23/7	14/9	9/9	15/9
Vin. 11	Anc.Nemea	350	10 - 20%	clay-loam	E - W	41B	3950	1/6	4/6	6/6	6/8	1/8	2/8	5/9	12/9	15/9
Vin. 12	Asprokampos	720	5 - 10 %	sandy-loam	N - S	R110	3780	10/6	8/6	10/6	4/8	5/8	5/8	24/9	25/9	25/9
Vin. 13	Asprokampos	710	0	sandy-loam	E - W	R110	3950	10/6	7/6	12/6	2/8	3/8	4/8	2/10	25/9	30/9
Vin. 14	Asprokampos	700	0 - 5 %	sandy-loam	E - W	R110	3950	10/6	7/6	12/6	2/8	3/8	4/8	2/10	25/9	30/9

*E-W refers to East West orientation, N-S to North-South.

***Vitis vinifera*

*** Vines per hectare

3.1.3 Vinification.

Red wine was produced from *Vitis vinifera* cv. Agiorgitiko grapes (20 kg from each vineyard) from Nemea region in Peloponnese in duplicate. In total 28 vinifications were performed per year (14 vineyards x 2 replicates). After crushing and destemming, 70 mg/l SO2 (as potassium metabisulfite) was added to the grapes and the must was transferred into 50 l stainless steel containers. Fermentations took place at the underground cellar of SEMELI winery in Koutsi (Nemea) under controlled ambient temperature (25°C). Lyophilized yeasts of the commercial strain Uvaferm NEM, (Lallemand, Grenaa, Denmark) at 20 g/hl (previously hydrated in water 15 min, 38 °C) and nutrients (Superstart, Laffort, Bordeaux, France) at 20 g / hl were added. Beginning on the next day and for the following 5 days the pomace was punched down twice a day, followed by two days of single punch down per day. The next day (8th day of maceration) juice was separated from the pomace and was left to complete fermentation for further three days (all fermentations were completed during that time.) Fermentation rate was daily recorded and completion of alcoholic fermentation was confirmed using the Rebelein method (Illand et al., 2004). After the completion of the alcoholic fermentation the wines were racked and supplemented with 50 mg/L SO2 (as potassium metabisulfite). No malolactic fermentation was performed. After a month, wines were bottled and stored at 18 ± 2 °C in the dark until analyzed. Wine analyses took place one month after bottling. Wine analyses were performed, every year, two months after bottling (January) while grape analyses on frozen grapes were performed 4-6 months after harvest.

3.2. Miscellaneous grape and wine analyses.

3.2.1 Miscellaneous analyses of grapes, grape skin and grape seeds.

At the time of harvest, a sub-sample of 300 berries was randomly selected and the essential analytical berry parameters were determined. Initially for each vineyard sample, berry weight and berry volume of one hundred 100 berries was measured. For berry volume a 1000 ml measuring cylinder was filled with exactly 500 ml of distilled water and one hundred (100) berries. Reading was recorded and the volume of water was subtracted. Berry weight of 100 berries was measured on an electronic scale. Total soluble solids were measured by hydrometry in Baume scale; pH was measured using a HANNA portable pH meter (HI 991003) and titratable acidity (TA) was measured by neutralization with sodium hydroxide, all methods according to Illand et al. (2000). Seed colorization (SC) was calculated by separating seeds from the pulp of the collected berries. Both surfaces (ventral and dorsal) were evaluated to determine and assign the color of the seeds to the color seed wheel created by Fredes et al., (2010). The overall seed coat color was obtained calculating the average between the ventral and dorsal surfaces. Finally, Botrytis infection was evaluated optically when the berries were harvested. If no infection was reported; sample was ranked as category A, if less than 5 % infection was recorded the sample was ranked as category B and in more than 5% infection the sample was ranked as category C.

3.2.2 Miscellaneous wine analyses.

Additional wine analyses were performed as descripted by Illand et al., (2000). PH was measured using a HANNA portable pH meter (HI 991003), titratable acidity was measured by neutralization with sodium hydroxide; alcohol

strength (% vol.) was measured by distillation. Color intensity was calculated as the sum of absorbance at 420, 520 and 620 nm and color hue as the ratio of 420 / 520 nm (Ribereau-Gayon et al., 2006). Measurement of total phenolic content was also performed as the absorbance at 280 nm. Finally, wine total dry matter was calculated according to OIV method MA-AS2-03B (2009).

3.3. Determination of phenolic compounds in grape skin and seed extract.

3.3.1 Extraction of phenolic compounds from grape skins and seeds.

Seeds and skins of 100 berries per triplicate were removed manually from grapes, freeze-dried and finally ground to powder. The extraction of skin and seed tannins was carried out according to previously reported methods (Kyraleou et al., 2015). More specifically, 3 gr of powder was added in 50 ml centrifuge tubes containing 25 ml of acetone/water solution (80/20) and stirred at 240 rpm for 3 hours at 20^{o}C. The tube was then centrifuged for 15 minutes at 8000 rpm at 20^{o}C and the supernatant was collected (Extract A). 25 ml of methanol/water solution (60 / 40) were added in the centrifuge tube containing the powder and stirred for 2.5 hours at 240 rpm at 20^{o}C. Finally the tube was centrifuged for 15 minutes at 8000 rpm at 20^{o}C and the supernatant was collected (Extract B). Both extracts were collected in the same glass vial, concentrated under reduced pressure at 30^{o}C and lyophilized to obtain dry powder. Glass vial weight was recorded before and after lyophilization. From the obtained powder model solutions were produced:

- Skin model solution: 100 ml solution 90 / 10 v.v. water / ethanol, 5 g/l tartaric acid, ph adjusted at 3.5, 2.5 g/l lyophilized skin powder.

- Seed model solution: 100 ml solution 90 / 10 v.v. water/ethanol, 5 g/l tartaric acid, ph adjusted at 3.5, 5 g/l lyophilized seed powder.

These model wines were further used for the determination of skin and seed phenolic compounds.

3.3.2 Determination of grape skin anthocyanins by High Performance Liquid Chromatography (HPLC).

0.5 g of lyophilized skin powder extract were brought into 50 ml centrifuge tube containing 20 ml of acidified methanol 0.1 HCL. The solution was covered with aluminum foil and stirred at 60rpm for 4 hours at 20^{o}C. Following extraction, the tube was centrifuged for 15 minutes at 8000 rpm at 20^{o}C and the supernatant was collected to a 50 ml glass vial. 10 ml of acidified methanol 0.1 HCL was added to the residue and the process was repeated for 18 hours extraction and the supernatant was collected in the same glass vial. Finally, the process was again repeated for 24 hours extraction and the supernatant was collected again to the same glass vial. The content of the glass vial was stirred well and filtered (0.2 μm) prior to injection to HPLC.

HPLC analysis was carried out for the determination of monomeric anthocyanins on a Restek pinnacle II C18 (250 x 4.6 mm, 5 μm) column at a flow rate of 1 ml / min, using a 10-μL injection volume, detection at 520 nm, and the following elution program: 90% eluent A for 1 min, then from 90% to 50% in 22 min and from 50% to 5% in 10 min, which was kept isocratic for further 2 min. Eluent A was 10% aqueous formic acid and eluent B methanol. Identification was based on comparing retention times and UV spectra of the peaks detected with those of original compounds. Malvidin-3-O-

acetylmonoglucoside (MlvAc) and malvidin-3-(6-Op-coumaroyl) monoglucoside (MlvCoum) were tentatively identified based on previous observations (Arnous et al., 2002; Kallithraka et al., 2005). Results were expressed as mg Mlv per g dry skin weight. All analyses were performed in triplicate. In Figure 3.1, is presented a chromatogram, where the 3-O-glucosides of delphinidin, cyanidin, petunidin, peonidin and malvidin, the malvidin-3-O-glucose acetate and malvidin-3-O-glucose coumarate are identified.

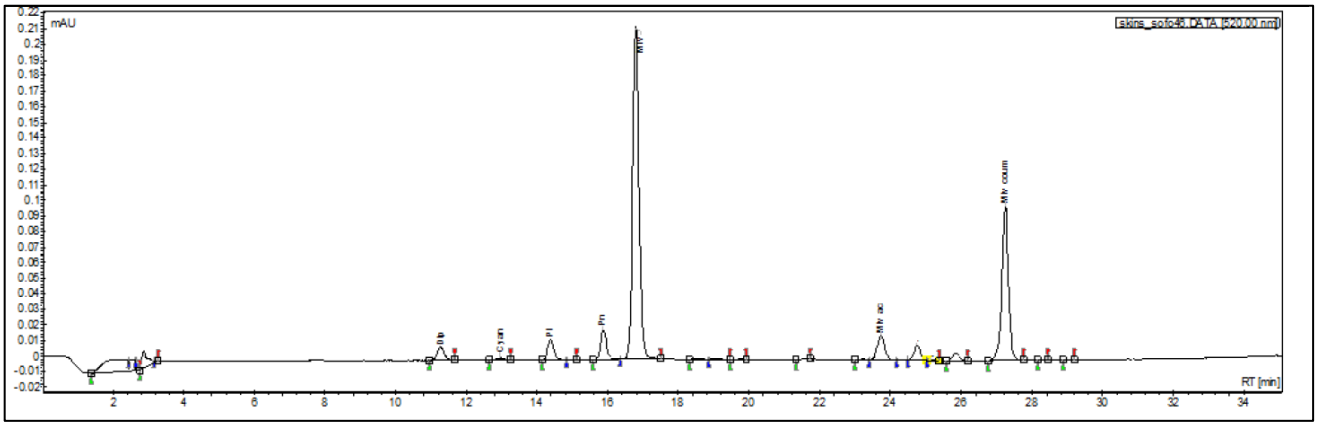


Figure 3.1: Chromatogram of anthocyanin HPLC analysis of grape skins extracts. *Dlp, Cyan, Pt, Pn* and *Mlv* stand for 3-O-glucosides of delphinidin, cyanidin, petunidin, peonidin and malvidin, respectively. *Mlv Ac:* malvidin-3-O-glucose acetate. *Mlv Coum:* malvidin-3-O-glucose coumarate. Calibration curve: y= 10,3x+7,5 mg anthocyanins in Mlv /L wine.

3.3.3 Mean degree of polymerization (mDP), percentage of galloylation (%G) and percentage of prodelphinidins (%P) of grape proanthocyanidins.

Tannin mDP and %G were determined in both organic and aqueous phases of seed and skin extracts. Tannin %P was determined only in skins. Tannin extracts were re-dissolved in methanol and were left to react with

phloroglucinol solution (50 g/l phloroglucinol, 10 g/l ascorbic acid, 0.1N HCl, in methanol) according to the method described by Chira et al. (2009).

Reaction products were analyzed by LC/MS on a Shimadzu LC/MS 2010A coupled to a single quadrupole mass spectrometer equipped with an electrospray ion source according to the method described by Kyraleou et al. (2015). The mass spectrometer was operated in positive-ion mode. The source's temperature was set at 70ºC, the capillary voltage at 3.5 kV and the cone voltage at -30 eV. The absorbance was recorded at 280 nm and mass spectra were recorded in the range of 50–1500 amu. Separation was performed on a reversed-phase Waters XTerra RR C18 (100 x 4.6 mm, 3.5 μm) column at a flow rate of 0.5 ml/min, using a 20-μl injection volume and the following elution program: eluent A from 80% to 40% in 20 min, which was kept isocratic for further 10 min and then from 40% to 80% in 2 min. Eluent A was 0.1% aqueous acetic acid and eluent B methanol. All analyses were performed in triplicate. In Figure 3.2, is presented a chromatogram of HPLC analysis of grape skin extracts where the polymers of proanthocyanidins (terminal and extension units) were identified.

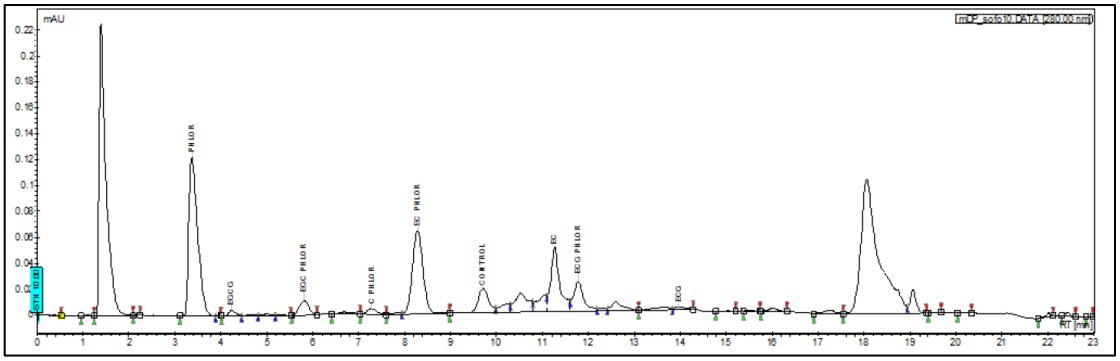


Figure 3.2: Chromatogram presenting HPLC analysis results of proanthocyanidins polymers of grape skin extracts. Terminal units and extension units bound with phloroglucinol were identified.

3.3.4 Grape phenolic parameters (Glories Method).

The method was developed by Glories and Augustin (1993) and is currently the most widely used method for color (anthocyanins) and phenolic composition of grapes. The principle of the method consists of rapidly extracting the anthocyanins and phenolic compounds from the skins and seeds, gently at first and then under more extreme conditions (Ribereau-Gayon et al., 2006). The difference between the results obtained reflects the extraction potential and can though used as ripeness indicator. The method is fairly easy, giving results that are both comprehensive and easy to interpret.

The procedure requires homogenizing of fifty (50) randomly selected berries in a blender (24.000 rpm for 30 s) and the extract is brought into 100ml flasks as shown in Table 3.2:

Table 3.2: Composition of initial grape extract solutions.

	Grape extract	pH=1	pH=3.6
Extract pH=1	20 g	20 ml	-
Extract pH=3.6	20 g	-	20 ml

Flasks were kept in dark for 4 hours and then centrifuged for 10 minutes at 4000 rpm at 20^{o}C and the supernatant was collected. Initially optical density at 280 nm (10 mm quartz cuvette, UV light lamp) was measured only at the Extract ph=3.6 supernatant. In Table 3.3 and 3.4, are shown the solutions that were prepared.

Table 3.3: Composition of the solutions prepared after the extraction of the phenolic compounds.

	Extract ph=3.6	Extract ph=1	EtOH / HCL 0.1%	Aqueous solution 2% HCL
Solution pH=1	–	1 ml	1 ml	20 ml
Solution pH=3.6	1 ml	–	1 ml	20 ml

Table 3.4: Composition of the solutions prior to measurement.

	Solution pH=1	Solution pH=3.6	H_2O	Na_2SO_3 15 %
pH=1 - H_2O	5 ml	–	2 ml	–
pH=1 - SO_2	5 ml	–	–	2 ml
pH 3.6 - H_2O	–	5 ml	2 ml	–
pH=3.6 - SO_2	–	5 ml	–	2 ml

After 20 minutes optical density at 520 nm (10mm glass cuvette, halogen lamp), was recorded in all samples. Initially the bleaching effect of SO_2 on the anthocyanin content was calculated with the equation:

A pH 1 = (OD_2-OD_1) x 885.3 / 1000 (g/l)

A pH 3.6 = (OD_2-OD_1) x 885.3 / 1000 (g/l)

Where: OD_1 = optical density of the sample containing Na_2SO_3 15 %.

OD_2 = optical density of the sample containing water.

From the above values and the optical density at 280 nm earlier recorded, the following calculations were conducted:

- Total anthocyanins: TA = ph1 (g/l)
- Anthocyanin extractability: AE % = (A ph 1 - A ph 3.6) x 100 / A pH 1
- Contribution of seed tannins to the total phenol content:

  MP % = [[A_{280} - (A ph 3.6 x 40)] / 1000] / A_{280} x 100
- Skin tannin concentration: Dpell = (ph 3.6 x 40) / 1000 (mg/l)

- Seed tannin concentration: Dpep = (A_{280} - dpell) (mg/l)

3.3.5 Total anthocyanins in red grape berries.

This method describes the measurement of total anthocyanins in red grape berries based on the methods described by Illand et al. (2000) and involves extraction with ethanol of these compounds from a known weight homogenized grape sample. A portion of the ethanol extract is then acidified at low pH and quantification based on the absorbance in visible region of the light spectrum. Malvidine is the major anthocyanin in *Vitis vinifera* grapes but is not the only and the results are expressed in malvidin-equivalents for comparative purposes only. In addition, measurement of the absorbance at 280 nm provides an estimate of the concentration of total phenolics in the solution.

The procedure requires homogenizing of fifty (50) randomly selected berries in a blender (24.000 rpm for 30 s). In 10 ml centrifuge tubes are brought 1 ml of grape extract and 10 ml of 50% Ethanol/H_2O solution. Tubes were covered with aluminum foil and stirred at 240rpm for 1 hour at 20_oC. Samples were then centrifuged (10 min at 4.000 rpm) and 0.5 ml of the extract was brought in glass tube containing 10 ml HCL 1N. Tubes were kept in dark for 3 hours and absorbance at 520 nm (1 mm glass cuvette, halogen lamp) and 280 nm (10mm quartz cuvette, UV light lamp) was recorded.

Anthocyanins mg/berry =

$$\frac{A_{520} \text{ x dilution factor x final extract volume (ml) x berry weight (g) x } 1000}{500 \text{ x } 100 \text{ x homogenate weight (g)}}$$

Anthocyanins mg/ gr of berry = Anthocyanins mg/berry / (weight of 50 berries/ 50)

Total phenolic (au): A_{280} x 100

3.4 Spectrophotometric analyses of phenolic compounds in grape extracts and in wine.

3.4.1 Folin-Ciocalteau assay.

The method is based on the fact that phenols ionize completely under alkaline conditions, and can be readily oxidized by the Folin-Ciocalteau reagent (Harbertson and Spayd, 2006). The oxidation causes a color change from yellow to blue easy to monitor with a spectrophotometer. The main drawback is that the Folin-Ciocalteau reagent is so reactive that it can also oxidize many unintended compounds in wine (like fructose, bisulfite, aminoacids, and ascorbic acid). In 10 ml volumetric flasks containing approximately 6 ml of distilled water are brought according to Table 3.5.

Table 3.5: Solution preparation for the Folin-Ciocalteau analysis.

	Wine or model wine	Folin-Ciocalteau	Na_2CO_3 20%
Extract	100 μl	0,5 ml	1.5 ml
Blanc	–	0,5 ml	1.5 ml

After 30 min the absorbance at 765nm (10 mm plastic cuvette, halogen lamp) was recorded with blank sample used as baseline absorbance. Calculation of the phenolic compounds concentration was performed through gallic acid standard curve. More specifically, 1 g/l gallic acid standard solution was made

and further dissolved to 50, 100, 250 and 500 mg/l solution. Absorbance at 765 nm of each solution was recorded and the curve gradient was calculated.

Total phenols (F - C assay): concentration of gallic acid equivalent x dilution factor (mg/l).

3.4.2 Adams-Harbertson (A-H) assay (BSA method).

The Adams-Harbertson tannin assay is a direct adaptation of a method previously used for grain and ecological tannin measurement (Hagerman and Butler, 1978) and is designed to be an inexpensive and reliable measurement of tannin in wine. The Adams-Harbertson assay utilizes protein precipitation with bovine serum albumin (BSA) and is used to quantify multiple classes of phenolic compounds: anthocyanins, tannins, pigmented polymers and non-tannin iron-reactive phenols. By combining protein precipitation and traditional bisulfite bleaching to distinguish monomeric anthocyanins from polymeric pigments, two classes of polymeric pigments in grapes and wines can be measured: small polymeric pigments (SPP) that do not precipitate with protein and large polymeric pigments (LPP) that do.

500 µl of wine or model wine are brought into 2 ml eppendorf tube, 1 ml of protein solution (BSA) was added and the tube was stirred for 15 min at 240 rpm at 20^{o}C. Sample was then centrifuged for 10 min at 12.000 rpm, the supernatant was discarded and 250 µl of acetic acid / NaCL solution was added to the residue. The tube was again centrifuged and supernatant was discarded. 875 µl of triethanolamine-sodium, dodecyl sulfate (TEA-SDS) was added to the residue and after 10 min tube was stirred with vortex. Absorbance at 510 nm (A1) was measured after 10 min (1mm cuvette,

halogen lamp, TEA-SDS solution as blank). Finally 125 µl $FeCl_3$ were added and after 10 min absorbance at 510 nm was measured (A_2). The difference A_2 - A_1 is the absorbance of tannin concentration. A standard curve of catechin standard solutions (25, 50, 100, 150, 200, 250, 300 mg/l) was created and the curve gradient was calculated.

Tannins (Adams-Harbertson, BSA): concentration of catechin equivalent x dilution factor (mg/l).

3.4.3 DPPH method and radical scavenging activity (%).

DPPH, known formally as 2, 2-diphenyl-1-picrylhydrazyl, is a cell-permeable, stable free radical that is commonly used to evaluate the ability of compounds to act as free radical scavengers or hydrogen donors and to measure the antioxidant activity of tissue extracts. The reaction of DPPH with an antioxidant or reducing compound produces the corresponding hydrazine DPPH, which can be followed by color change from purple (absorbance at 515-528 nm) to yellow. It is a rapid, simple, inexpensive and widely used method to measure the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity of foods. DPPH radical-scavenging activity was performed by the method described by Akter et al., (2010). For each determination, in 2 ml eppendorf tubes were mixed 25 µl of wine or model wine and 975 µl of DPPH standard solution and the absorbance at 515 nm (1 mm cuvette, halogen lamp) was recorded (A_0). Sample was returned into the eppendorf tube and measurement was repeated after 30 min (A_{30}). Standard trolox solutions were prepared (0.08, 0.5, 1.0, 1.5.

and 2 mM trolox) and the percentage of DPPH scavenging versus concentration of samples was plotted.

DPPH radical scavenging activity (%) = $[(A_0 - A_{30}) / A_0] \times 100$

Finally, through the plotted curve the radical scavenging activity expressed as mM trolox was calculated.

3.4.4 Tannin measurement by acid hydrolysis.

It is a method that has been commonly used for a long time and exploits the ability of the tannin molecules to break down in a heated acid environment (acid hydrolysis method, Ribereau-Gayon and Stonestreet, 1965). The individual molecules show a red coloration after the heating process and can then be measured by quantifying the intensity of the red tonality using a conventional spectrophotometer. This method that is used worldwide presents a number of limitations. It does not take into account the structure of the tannin pool and it also does not consider other components (e.g. anthocyanins) that can interfere in the reaction and measurement. Due to this, the tannin concentration in wine is often overestimated and it is common to observe an increase in the wine total tannin content during ageing. Nevertheless, the method also has some advantages as the ease of implementation and reliability.

In a pair of glass tubes are brought 2 ml of wine or model wine, 1ml of distilled water and 3 ml of HCl 37%. One tube (A_2) is heated at 100^{o}C for 45 min while the other (A_1) was kept at room temperature. The heated tube is then brought to room temperature and 500 µl of ethanol (95%) were added into both tubes.

Optical density at 550 nm was recorded at both samples (10mm cuvette, halogen lamp) and the total tannin content (gr/lt) was calculated:

Tannins = (A_2-A_1) x 19.35 (g/l).

3.4.5 DMAC Index.

Monomeric flavanols can be measured with the aldehydic reagent, 4-dimethylaminocinnamaldehyde (DMAC), which reacts with the aromatic ring on all free meta-hydroxyl groups on the A-ring in an acidic medium to determine monomeric flavan-3-ols, as described by Nagel and Glories (1991). Due to this mechanism, proanthocyanidins are also included in this measurement, but react with DMAC to a much lesser extent than monomeric flavan-3-ols. Anthocyanins and flavonols are excluded due to their electron-withdrawing functional groups.

In glass tubes were brought wine or model wine according to Table 3.6.

Table 3.6: Solution preparation for the DMAC index.

	Wine or model wine	Methanol	DMAC
Extract	40 μl	4.7 ml	200 μl
Blanc	40 μl	4,9 ml	-

Both tubes were stirred vigorously and optical density at 640 nm (10mm glass cuvette, halogen lamp), was recorded after 15 min. The difference in absorbance was calculated and standard catechin solutions were prepared (0.08, 0.5, 1.0, 1.5. and 2 mM trolox) and measured. DMAC values versus concentration of samples were plotted and further calculated the mg/l of catechin equivalents.

3.4.6 Methyl cellulose precipitable tannin assay (MCP).

The MCP (methyl cellulose precipitable) tannin assay is a simple and robust means of measuring the total grape or wine tannin in red grape homogenate extracts and red wine (Sarneckis et al., 2006). The assay is based upon polymer-tannin interactions resulting in the formation of insoluble polymer tannin complexes which then precipitate. The method requires a control sample (i.e. no methyl cellulose added) and a treatment sample to be prepared. The A_{280} value of the control sample indicates the value for all phenolic compounds (total phenolic), whereas the A_{280} value of the treated sample indicates the value for phenolic compounds remaining in solution after the MCP tannin has precipitated. By subtracting these two values, the A_{280} of the MCP tannin in a solution can be determined and then related to epicatechin equivalents or used as an arbitrary value.

In 10 ml centrifuge tubes are brought 1 gr of homogenized grapes and 10 ml of 50% ethanol / H_20 solution. Tubes are then covered with aluminum foil and stirred at 240 rpm for 1 hour at 20^oC. In the case of wine no extraction is required. Samples were centrifuged (for 10 min at 4.000 rpm) and in new centrifuge tubes the solutions presented in Table 3.7 were made (volume of sample was adjusted accordingly in the case of wine or grape extract):

Table 3.7: Methyl cellulose precipitable tannin assay.

Grape extract					
	Final volume	Supernatant	Methyl cellulose	Ammonium phosphate	H_2O
Control	10 ml	1 ml	–	2 ml	7 ml
Treatment	10 ml	1 ml	3 ml	2 ml	4 ml

Wine					
	Final volume	Supernatant	Methyl cellulose	Ammonium phosphate	H_2O
Control	10 ml	250 µl	–	2 ml	7,75 ml
Treatment	10 ml	250 µl	3 ml	2 ml	4,75 ml

Samples were again centrifuged (10 min at 4.000 rpm) and absorbance at 280 nm (10 mm quartz cuvette, UV light lamp). The difference between the absorbance values ($A_{Control}$–$A_{Treatment}$) was calculated and converted to mg/lt catechin (C_{Tannin}) using a standard catechin solution plotted curve (10, 50, 100, 150, 200 mg/l catechin).

For wine:

Concentration of tannins: C_{Tannin} x 10 (mg/l catechin)

For grape extract:

Concentration of tannins: C_{Tannin} x 10 x Ve / Wh (mg/l catechin)

Where: Ve = final volume of extract (0.0105 l)

Wh: weight of homogenized sample used for extraction (g).

3.5 Amino acid analyses of grape must and wine.

The concentration of individual AA was determined by high performance liquid chromatography (HPLC) after derivatization with O-phthalaldialdehyde (OPA) according to the method described by Bena-Tzourou, (1999). Initially the frozen grapes (-20^{o}C) were crushed and grape juice was extracted and filtered through No. 4 Whatman paper and then diluted (1:10 v/v) with internal standard solution (Norvaline) at 62.5 mmole/l in 0.1 M HCl. This solution was

again filtered through a disposable 0.45-mm filter before it was injected into the chromatographic column. Wine samples were prepared in the same way, but excluding the initial step of filtration. The autosampler was programmed to add 5-mL sodium borate buffer (0.4N, pH 10.4) to 1-ml OPA reagent and 1-ml sample. The reaction mixture was then agitated during 6 cycles (10 sec/cycle) before adding 1-ml FMOC-Cl reagent. The mixture was agitated during 3 cycles and finally injected (injection volume was 8 mL). The chromatographic system was Hewlett Packard 1090 Series II/M AminoQuant liquid chromatograph including: column oven, binary eluent system DR5, autosampler; a programmable Hewlett Packard 1046A fluorescence detector, Hewlett Packard 9153C drive, Hewlett Packard ChemStation 9000/300 and Hewlett Packard Think Jet printer. The chromatographic column was a narrow bore C18 HP AminoAcid Analysis (200 x 2.1 mm), protected by a 15 × 2.1 mm guard column. Eluent A was a 20 mM sodium acetate buffer (pH 7.2 adjusted with acetic acid solution, 2% v/v), containing 0.018% v/v triethylamine. Eluent B was a 20% 100 mM sodium acetate buffer (pH 9.10 adjusted with acetic acid diluted 2% v/v), 40% acetonitrile and 40% methanol. Flow gradient conditions are presented into Table 3.8 (Godel et al., 1991).

Table 3.8: Flow gradient program of primary amino acid determination by HPLC.

Time (min)	Eluent A%	Eluent B%	Flow rate (ml/min)
0.0	100	0	0,45
17.0	40	60	0,45
18.0	0	100	0,45
18,1	0	100	0,45
18,5	0	100	0,80
23,9	0	100	0,80
24,0	0	100	0,45
25,0	0	100	0,45

Excitation/emission wavelengths were respectively 340/450 nm and the free amino acid quantification was performed by the internal standard method with norvaline (primary amino acid internal standard). Peaks were identified by comparison of retention times with commercial standards (L-Amino acids Kit) purchased from Sigma-Aldrich (Germany) (Figure 3.3).

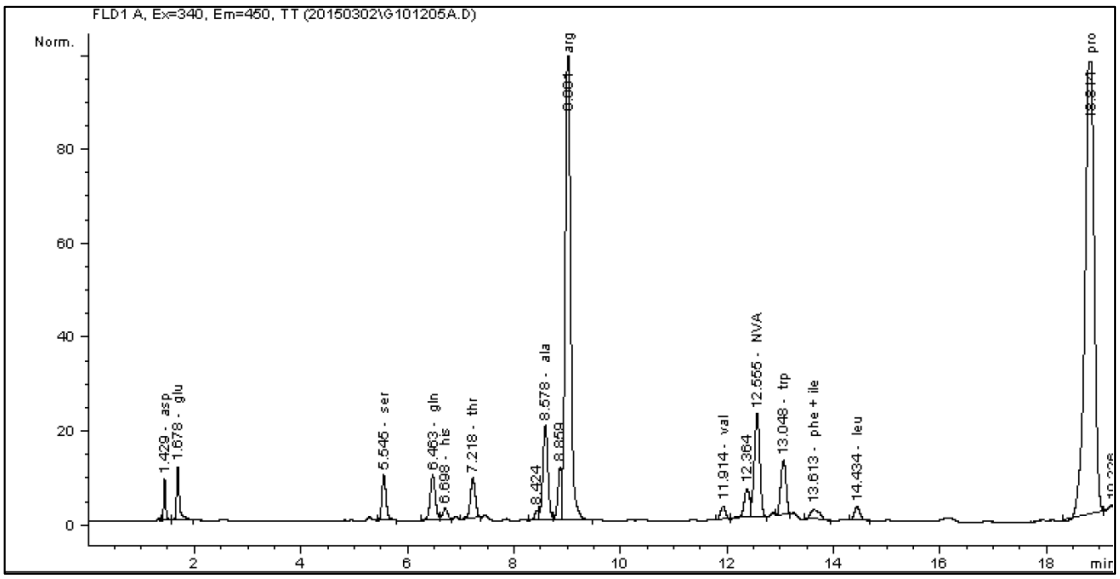


Figure 3.3: Chromatogram presenting HPLC analysis results of amino acid analysis.

Free amino acid nitrogen fraction (FAN) or yeast assimilable nitrogen (YAN) was also measured according to the method descripted by Dukes and Butzke (1998). All analyses were performed in duplicate. Calibration curves are presented in the `Appendices` section.

3.6. Wine analyses.

3.6.1 Mean degree of polymerization (%mDP), percentage of galloylation (%G) and percentage of prodelphinidins (%P) of wine proanthocyanidins.

In wines isolation of proanthocyanidins took place using a C18 (Lichrolut C18, 5 g octadecyl bonded endcapped silica, 25 ml volume) SPE cartridge according to the method described by Chira et al., (2012). The cartridge was

initially activated adding sequentially 25 ml methanol, 25 ml distilled water and finally the diluted wine extract. The cartridge was then washed with 50 ml distilled water and left to dry for 15 minutes. Elution of the proanthocyanidins was performed with 50 ml methanol and the tannin extracts were evaporated under reduced pressure at 30ºC and lyophilized to obtain a dry powder. Tannin extracts were re-dissolved in methanol and were left to react with phloroglucinol solution and analyzed by LC-MS as previously described. Calibration curves are presented in the `Appendices` section.

3.6.2 HPLC determination of wine anthocyanins.

Wine samples were filtered through 0.45 μm syringe filters prior to High Pressure Liquid Chromatographic (HPLC) analysis (Hewlett-Packard 1050) using a HP 1050 chromatography apparatus coupled to a diode array detector. Analyses were performed as in Kallithraka et al. (2005) on a Spherisorb ODS-2 column (particle size, 5 μm; 250 x 4 mm id), at a flow rate of 1 ml min-1, using a 20 μl injection volume, detection at 520 nm, and the following elution programme: 95% eluent A for 1 min, then from 95 to 50% in 25 min, and finally from 50 to 5% in 3 min, which was kept isocratic for another 3 min. Eluent A was 10% aqueous formic acid and eluent B was MeOH (HPLC grade, Sigma). Identification was based on comparing retention times of the peaks detected with those of original compounds and on UV-vis on- line spectral data. Malvidin-3-O-glucose coumarate (MvCoum) and malvidin-3-O-glucose acetate (MvAcet) were tentatively identified based on previous observations (Arnous et al., 2002). All peaks were quantified as malvidin-3-O-glucose (Mv) (Extrasynthèse, France). In Figure 3.4 is presented a chromatogram, where the 3-O-glucosides of delphinidin, cyanidin, petunidin, peonidin and malvidin,

the malvidin-3-O-glucose acetate and malvidin-3-O-glucose coumarate are identified.

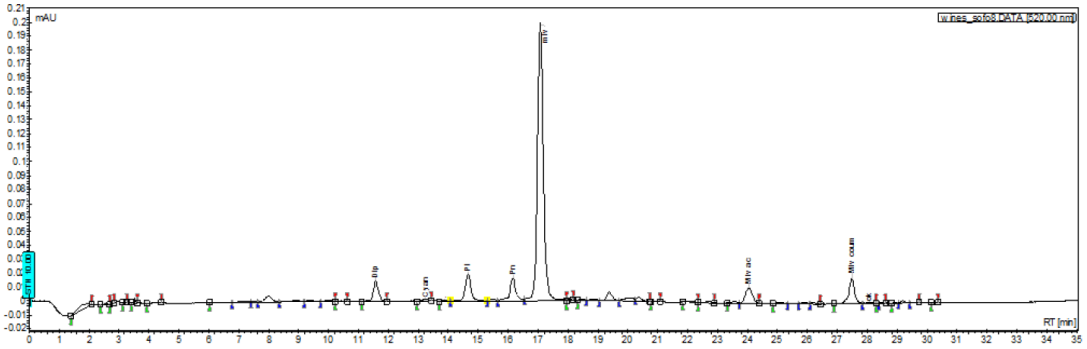


Figure 3.4: Chromatogram of anthocyanin HPLC analysis of wine. *Dlp, Cyan, Pt, Pn* and *Mlv* stand for 3-O-glucosides of delphinidin, cyanidin, petunidin, peonidin and malvidin, respectively. *Mlv Ac:* malvidin-3-O-glucose acetate. *Mlv Coum:* malvidin-3-O-glucose coumarate. Calibration curve: y= 10,3x+7,5 mg anthocyanins in Mlv /L wine.

3.6.3 Modified Sommers wine analysis.

The principle of this spectroscopic analysis is based on the methods of Somers and Evans (1977). The Somers color assay was modified to allow the standardization of pH and ethanol concentrations of wine samples in a simple one-step dilution with a buffer solution, thus removing inconsistencies between wine matrices. Red wine color measurements are based on the absorbance of monomeric anthocyanin pigments and polymeric pigment forms in the visible and ultraviolet regions. The method is a set of spectroscopic colour measurements, which not only give a measure of wine colour but also give an insight into the contributing elements such as anthocyanin equilibria and phenolic composition. Measures of wine color density, wine color hue, total phenolic, total anthocyanins, degree of ionization of anthocyanins and free and molecular sulfur dioxide are included in this profile.

In glass tubes were brought the following solutions:

- 100 µl wine, 4.9 ml HCl 1 M. Tubes were stirred vigorously and kept in dark for 3 hours. Absorbance was then recorded at 280 nm (A_{280HCL}), 420 nm ($A_{420\ HCL}$) and 520 nm ($A_{520\ HCL}$).
- 500 µl wine, 4.5 ml 0.1 % acetaldehyde-model wine solution (BFS). Tubes were stirred vigorously and kept in dark for 1 hour. Absorbance was then recorded at 420 nm ($A_{420\ ACET}$), 520 nm ($A_{520\ ACET}$) and 620 nm ($A_{620\ ACET}$).
- 500 µl wine, 4.5 ml 0,375% Sodium metabisulfite -model wine solution (BFS). Tubes were stirred vigorously and kept in dark for 1 hour. Absorbance was then recorded at 420 nm ($A_{420\ SULF}$) and 520 nm ($A_{520\ SULF}$).
- 500 µl wine, 4.5 ml model wine solution (BFS). Tubes were stirred vigorously and instantly absorbance was recorded at 420 nm (A_{420} BFS), 520 nm (A_{520} BFS) and 620 nm (A_{620} BFS).

Using the obtained measurements the following calculations were conducted:

- Chemical age (Index of chemical age 1): $A_{520\ SULF} / A_{520\ ACET}$
- Chemical age (Index of chemical age 2): $A_{520\ SULF} / (5 \times A_{520\ HCL})$
- Degree of ionization of anthocyanins after abolishing SO2 effect on wine color:

$$\frac{(10 \times A_{520\ BFS}) - (10 \times A_{520\ SULF})}{(50 \times A_{520\ HCL}) - [1.6667 \times (10 \times A_{520\ SU}} \times 100 \quad (\%)$$

- Total anthocyanins: 20 x [(50 x $A_{520\ HCL}$) - (1.6667 x (10 x $A_{520\ SULF}$))] (mg/l)
- Color density: ($A_{420\ BFS}$ + $A_{520\ BFS}$ + $A_{620\ BFS}$) x 10 (au)
- Color density corrected from SO_2 bleaching: $A_{420\ ACET}$ + $A_{520\ ACET}$ + $A_{620\ ACET}$) x 10 (au)
- Total phenolic: ($A_{280\ HCL}$ x 50) - 4 (au)
- Color resistant to SO_2 bleaching: $A_{520\ SULF}$ x 10 (au)

3.7 Sensory evaluation.

3.7.1 Training of sensory panel.

Eleven panellists participated in this experiment. All of them were professionally trained and selected based on their experience in vinification of Agiorgitiko with minimum vinification/working experience in Nemea region of ten years. Three training sessions took place to familiarize the panel with the tasting procedure. The panellists were instructed to use and define the criteria which describe and discriminate the sensory parameters they had to assess. For scoring, a modified version of the Davis scorecard was used (Table 3.9). The scorecard was firstly developed by the Department of Enology and Viticulture at the University of California and is used extensively by the wine industry since then.

It is easily adapted by panellists and provides reliable results easily interpreted (Amerine and Roessler, 1983). The scorecard has been modified, since the attributes of the original version are inadequate to describe the complexity of current wines (Winiarsky et al., 1997). The attributes which

tasters had to access were appearance, aroma, acidity, flavour, balance, development, finish and overall quality.

Table 3.9: Modified version of the Davis score sheet.

Appearance and colour	
0	POOR - Dull or slightly off-colour
1	GOOD - Bright with characteristic colour
2	SUPERIOR- Brilliant with characteristic colour
Aroma and bouquet	
0	OFF CHARACTER - Marginal expression of an off-odour
1	ACCEPTABLE - No characteristic varietal-regional-stylistic fragrance or aged bouquet
2	PLEASANT - Mild varietal-regional-stylistic fragrance or aged bouquet
3	GOOD - Standard presence of a varietal-regional-stylistic fragrance or aged bouquet
4	SUPERIOR - Varietal-regional-stylistic fragrance or aged bouquet distinct and pleasant
5	EXCEPTIONAL - Varietal-regional-stylistic fragrance or aged bouquet rich, complex, refined
Acidity	
0	POOR - Acidity either too high (sharp) or too low (flat)
1	GOOD - Acidity appropriate for the wine style
Balance	
0	POOR - Acid/sweetness ratio inharmonious, excessively bitter and astringent
1	GOOD - Acid/sweetness ratio adequate; moderate bitterness and astringency
2	EXCEPTIONAL - Acid/sweetness balance invigorating; smooth mouth-feel
Development/duration	
0	POOR - Fragrance simple, does not develop, of short duration
1	STANDARD - Fragrance typical, develops in complexity, does not fade during tasting
2	SUPERIOR - Fragrance improves in intensity and/or character, lasts throughout tasting
3	EXCEPTIONAL - Rich fragrance, improves in intensity and character, long lasting
Flavour	
0	FAULTY - Off-tastes or off-odours so marked as to make the wine distinctly unpleasant
1	POOR - Absence of varietal, regional or stylistic flavour characteristics
2	GOOD - Presence of typical varietal, regional, or stylistic flavour characteristics
3	EXCEPTIONAL - Superior expression of varietal, regional or stylistic characteristics
Finish	
0	POOR - Little lingering flavour in the mouth; excessive astringency and bitterness
1	GOOD - Moderate lingering flavour in the mouth, pleasant aftertaste
2	EXCEPTIONAL - Prolonged flavour in the mouth (>10 to 5 s), delicate and refined aftertaste
Overall quality	
0	UNACCEPTABLE - Distinctly off-character
1	GOOD - Acceptable representation of traditional aspects of wine type
2	SUPERIOR - Clearly better than the majority of the wines of the type
3	EXCEPTIONAL - So nearly perfect in all sensory qualities as to be memorable

The training period included a general session where the panellists were presented with the samples they had to assess and asked to develop the criteria concerning the use of the scale for each sensory attribute. After they had agreed on the sensory characteristics of a typical young red Agiorgitiko wine (PDO Nemea), in order to have a uniform performance during the next two sessions they were asked to evaluate a selection of typical Agiorgitiko wines and further discuss and justify whether the wines were of low, medium or high grade in the specific attribute ratings.

3.7.2 Tasting procedure.

After receiving the appropriate training, the panellists evaluated each wine in triplicate. The tastings were conducted from 11:00 to 13:00 in room temperature in individual booths. The samples were presented using a balanced block design in coded wine glasses, to balance the effect of presentation order. In each session, seven samples were evaluated over 30 minutes and a 10 min break was taken between the samples, while the panellists were asked to wash their mouths with water. Tasters were asked to evaluate and score the wines according to the modified `Davis` scoring sheet, values obtained for each attribute were summed and mean scores were calculated. Each year's wines were tasted four times; two tasting sessions took place 4-8 weeks after bottling while the other two took place 16-20 weeks after bottling. The results of each session were summed and statistical analysed. None of the wine samples presented, exhibited vinification defects or taint.

3.8.2 Fuzzy logic decision making system.

3.8.1 Introduction to fuzzy logic.

Real-world decision-making problems are usually too complex and ill-structured to be considered through the examination of a single criterion that will lead to the optimum decision (Kachraman et al., 2015). Multicriteria Decision Making (MCDM) constitutes an advanced field of operations research that is devoted to the development and implementation of decision support tools and methodologies to confront complex decision problems involving multiple criteria, goals, or objectives of conflicting nature (Zoupounidis and Doumpos, 2002). In various situations of daily life; for evaluation, judgment, and decision, natural language is often employed in order to articulate thinking and subjective perceptions. In such cases, words might not have a clear and well-defined meaning. As a result, if the word is used as a label for a set, the boundaries of the set to which objects do or do not belong will become fuzzy (Mardani et al., 2015).

The concept of fuzzy logic was first introduced in 1965 by Professor Lotfi Zadeh, from the Faculty of Electrical Engineering, (U.C. Berkeley). Fuzzy logic is a logic operations method based on many-valued logic rather than binary logic (two-valued logic). Two-valued logic often considers 0 to be false and 1 to be true. However, fuzzy logic deals with truth values between 0 and 1, and these values are considered as intensity (degrees) of truth (Figure 3.10). Currently, fuzzy logic decision making systems are widely used as decision methodologies in engineering, technology, science and management and business.

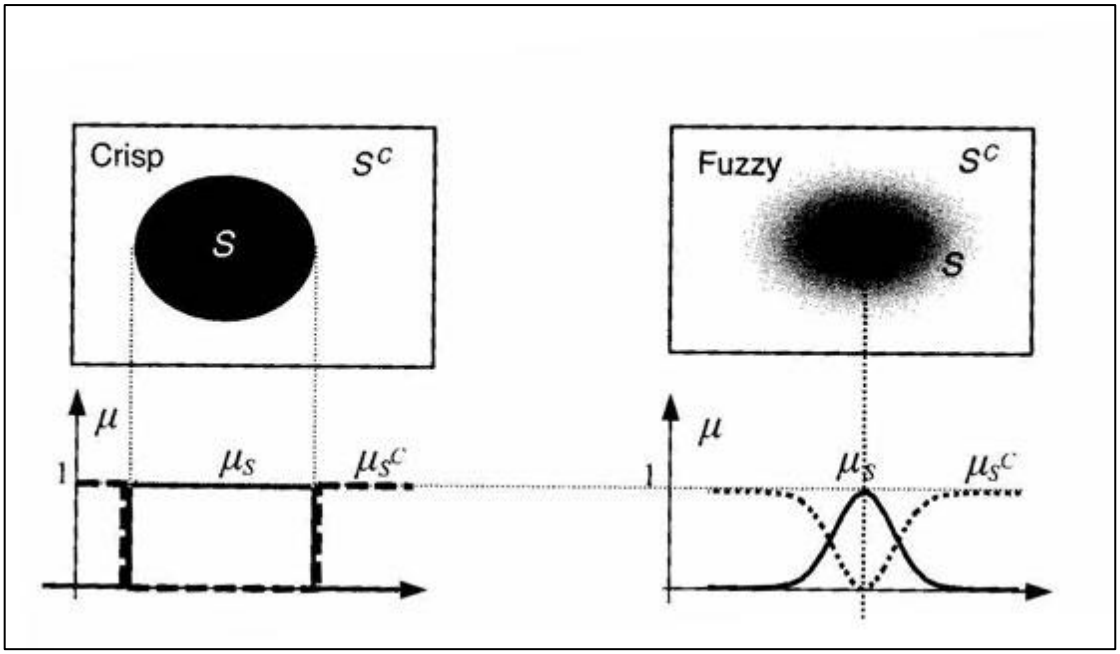


Figure 3.5: Graphical representation of a conventional set (left) and a fuzzy set (right).

3.8.2 Concept.

Let X be the universe of discourse and its elements denoted by x. A fuzzy set A is characterized by a membership function $g_a : X \to [0,1]$. A real fuzzy number can be described as any fuzzy set A of real numbers and its degree of membership can be defined with the following membership function (Dubois and Prade, 1978; Bortolan and Degani, 1985).

$$g_A(x) = \begin{cases} g_L(x), & a \le x \le b \\ 1, & b \le x \le c \\ g_{R(x)}, & c \le x \le d \\ 0, & \text{otherwise} \end{cases}, A = \{(x, g_a(x)) / x \in X\}$$

The shape of the membership function is usually chosen based on several studies or by the experts. The triangular and trapezoidal fuzzy number constitute the most commonly used fuzzy numbers and can be donated as

$A=(a,d,c)$ and $B=(a,b,c,d)$, respectively. Their membership functions can be expressed as:

$$g_A(x)=\begin{cases}\left(\dfrac{x-a}{b-a}\right), & a\le x\le b\\ \left(\dfrac{c-x}{c-b}\right), & b\le x\le c\\ 0, & \text{otherwise}\end{cases}$$

Where $x\in A, g_A(x):R\to[0,1]$ is the membership function of the triangular fuzzy number.

$$g_B(x)=\begin{cases}\left(\dfrac{x-a}{b-a}\right), & a\le x\le b\\ 1, & b\le x\le c\\ \left(\dfrac{d-x}{d-c}\right), & c\le x\le d\\ 0, & \text{otherwise}\end{cases}$$

Where $x\in B, g_B(x):R\to[0,1]$ is the membership function of the trapezoidal fuzzy number.

3.8.3 Building the linguistic variables set and rules.

Various situations cannot be described by traditional quantification methods, because they contain complicated conditions. The values of a linguistic variable are words or sentences which express the human knowledge and information. These linguistic values can be expressed as “poor, good, excellent, etc.” In a fuzzy system, typical fuzzy rules can correspond to the decision making process. The typical fuzzy rules utilize the if-then else rules and a problem with N data have the following form (Kuncheva, 2000):

$$If: x_1 \text{ is } A_{i1} \text{ AND } x_2 \text{ is } A_{i2} \text{ AND} ... x_n \text{ is } A_{in} \text{ THEN } D_i$$

Where $D = (D_1, D_2, D_3, ..., D_k)$ are the K decisions for a N data decision problem, $x \in X \subseteq R^n, x = (x_1, x_2, x_3, ..., x_n)$ and A_{i1} represents the fuzzy set.

3.8.4 Structure of the fuzzy interface system.

A fuzzy interface system is based on fuzzy set theory in order to define the input values to outputs. The fuzzy interface system involves four steps (Nasiri et al., 2007): *(a) Fuzzification step*. In this step, the input values are concerned into linguistic values using the membership functions; *(b) Rule assessment step*. In a multiple input – single output system (MISO), the rules are estimated according to their importance. Selecting the number of rules is a typical trade-off between model accuracy and complexity (Haber and Unbehauen, 1990); *(c) Aggregation of rules outputs step*. In this step, the outputs are aggregated into a single fuzzy distribution. The output is calculated based on the degree of activation of the rules; *(d) Defuzzification step*. The output fuzzy set is mapped into a crisp number. There are several methods for defuzzification such as “centroid”, “maximum”, “mean of maxima”, “height” and “modified height”. The centroid method is the most popular defuzzification method and it was selected to be used in this work. The centroid method was used to calculate the centre of gravity of the membership function for the fuzzy set (Kasabov, 1998). The model that was designed for this application is shown in Figure 3.6.

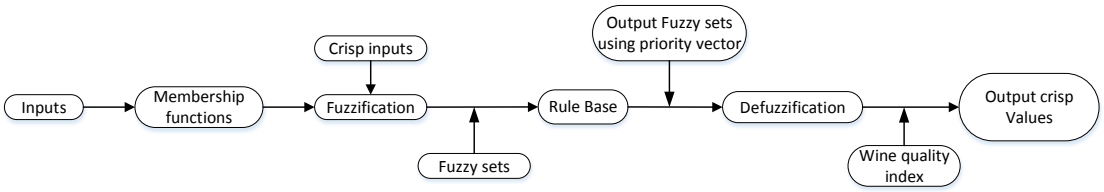


Figure 3.6: The Fuzzy logic tool structure.

3.9 Statistical analysis.

Analysis of variance (ANOVA) was performed using Statistica V.7 (Statsoft Inc., Tulsa, OK, USA) to determine whether the mean values of the sensory parameters differed between treatments and the replicates. All chemical analysis were performed in triplicates or otherwise indicated. Tukey's HSD test were used as comparison tests when samples were significantly different after ANOVA ($p<0.05$) for chemical and sensory analysis respectively.

Results and discussion.

4.1 Harvest year.

Meteorological data were collected during the three years of the experiment from the meteorological station positioned at the Cooperative Winery of Nemea, near the city of Nemea at 290 m altitude. Data were collected in daily basis and calculation of mean monthly temperature, monthly rainfall, annual heating degree days and annual growing degree days was performed, for all years of the experiment (Figure 4.1).

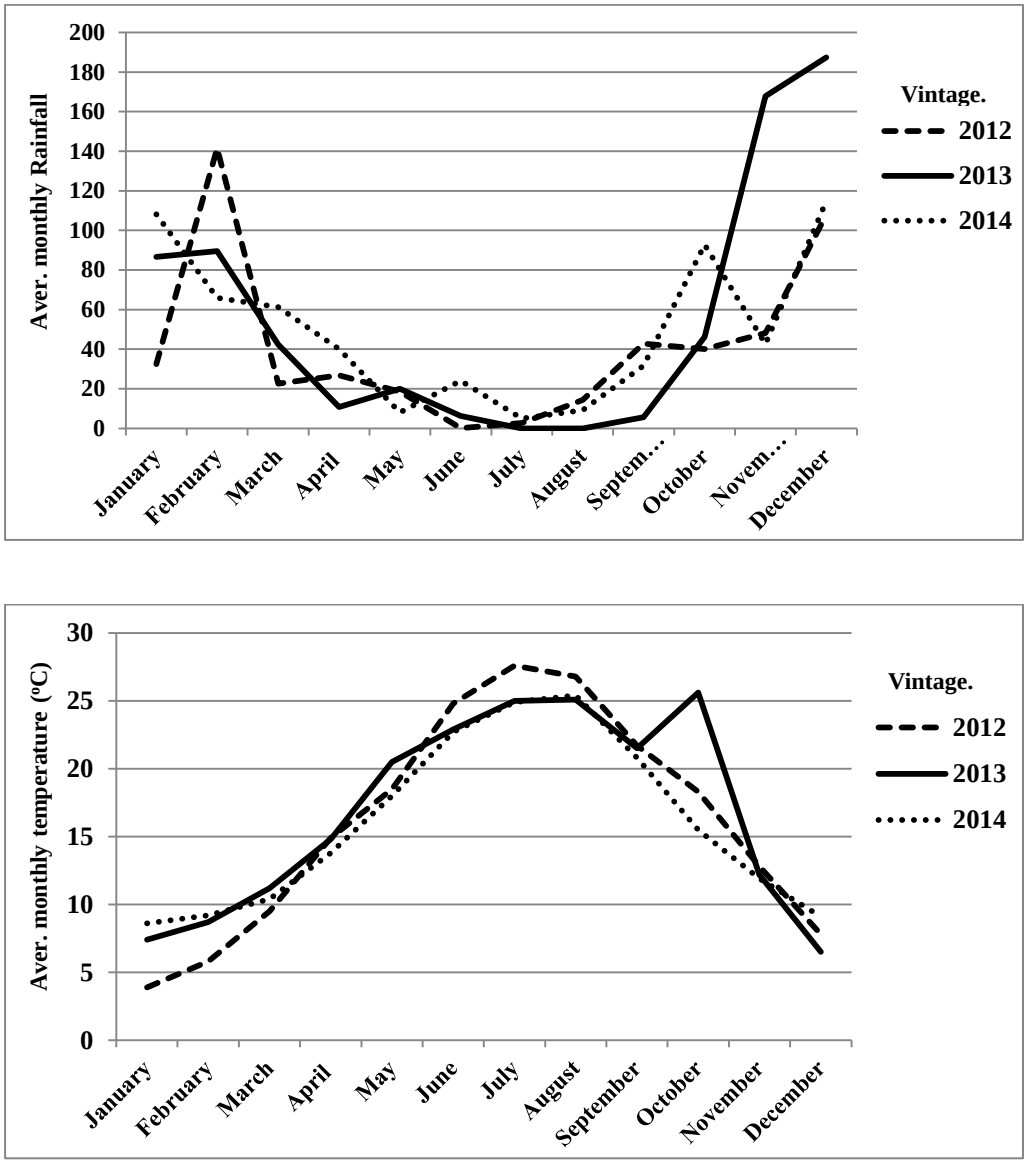


Figure 4.1: Average monthly rainfall (top) and average monthly temperature (bottom), during the three consecutive years of the experiment.

The growing season of 2012 was characterized by elevated temperatures from flowering until harvest in comparison to 2013 and 2014. During harvest, heavy rainfall occurred resulting to heavy botrytis load, and reduced grape quality. Grapes were sensory evaluated every two days (data not shown) focused on phenolic maturity as indicated by seed colorization (Fredes et al., 2010). Even though grape sugar maturity was reached, phenolic maturity was not; resulting to unbalanced wines compared to vintage 2013 and 2014 (Table 4.1). Unlike the growing season of 2012 and 2014, 2013 was a cool season with low average temperatures during vegetative growth, slow maturation, lack of rainfall during harvest and minimum botrytis infection. Due to the weather conditions sugar maturity coincided with phenolic maturity. Finally, growing season 2014 was characterized by increased rainfall during flowering and fruit set, causing extended downy mildew infection to the region. From flowering to harvest, temperatures remained low, giving place to intense rainfall during harvest resulting in extended botrytis infection.

Table 4.1: Meteorological data during the three consecutive years of the experiment.

Vintage 2012	January	February	March	April	May	June	July	August	September	October	November	December	**Average**	**Sum**
Mean C	3.9	5.8	9.5	14.9	18.5	24.8	27.6	26.8	21.7	18.3	12.8	7.8	**16.0**	
HDD*	447.4	362.4	277.5	129.3	69.3	15.7	3.9	5.6	31.0	71.6	173.4	318.4		**1905.5**
Rain mm	32.5	141.4	22.6	26.8	18.6	0.2	2.8	14.4	42.8	40.0	48.2	108.0		**498.3**
GDD**				114.0	260.4	427.5	528.6	492.9	384.0	268.2				**2475.5**
Vintage 2013	January	February	March	April	May	June	July	August	September	October	November	December	**Average**	**Sum**
Mean C	7.4	8.7	11.2	14.8	20.5	22.9	25.0	25.1	21.5	25.6	12.2	6.5	**16.8**	
HDD*	303.8	261.6	224.3	142.6	44.7	25.8	9.1	9.9	36.6	126.3	190.7	367.7		**1743.1**
Rain mm	86.6	89.6	42.4	10.8	20.0	6.2	0.0	0.0	5.6	46.2	167.8	187.4		**662.6**
GDD**				175.5	325.5	360.0	486.7	489.8	339.0	173.6				**2350.1**
Vintage 2014	January	February	March	April	May	June	July	August	September	October	November	December	**Average**	**Sum**
Mean C	8.6	9.2	10.4	13.8	18.0	22.7	24.9	25.4	20.8	15.5	11.9	9.1	**15.9**	
HDD*	302.4	258.1	250.9	150.9	80.4	23.3	8.2	6.8	32.3	123.4	194.6	286.7		**1718.0**
Rain mm	108.2	66.0	61.2	40.0	8.0	24.0	5.0	9.2	31.6	92.8	42.6	114.7		**603.3**
GDD**				126.0	237.2	424.5	452.6	477.4	309.0	255.8				**2282.4**

*HDD: Heating degree days.

**GDD: Growing degree days.

4.2 Grape and juice analyses.

4.2.1 Grape and juice general analyses.

Classical analyses were performed on grapes and juice and the results are summarized in Table 4.2. Berry weight and berry volume values varied between 1.59 to 2.56 g and 1.40 to 2.38 ml respectively. The pH values varied between 3.17 and 4.18 and titratable acidity values were between 3.7 and 8.6 expressed as g/l tartaric acid. Sugar content of grapes expressed as Baume degrees ranged from 11.00 to 14.5 while Botrytis infection was observed only in few vineyard samples and only for vintages 2012 and 2014. Finally seed colorization according to Fredes et al. (2010) was variable.

Table 4.2: Grape and juice analyses. Values are the means of triplicate determinations.

Vintage 2012							
Code	**Berry Weight**	**Berry volume**	**Baume**	**Titratable acidity**	**pH**	**Botrytis inf.**	**Seed color.**
Vin 1	2.05±0.1 f,g	1.88±0.04 b,c	14.1±0.2 a,b	5.1±0.3 e	3.74	0	0
Vin 2	2.01±0.3 g	1.83±0.04 b,c	14.4±0.1 a	4.9±0.1 f	3.86	0	0
Vin 3	2.46±0.4 c	2.30±0.02 d,e	13±0.2 c,d,e	4.6±0.2 g	3.84	0	1
Vin 4	1.96±0.2 e,f	1.82±0.02 b	12.8±0.1 d,e	5.3±0.1 e	3.77	0	1
Vin 5	2.47±0.1 c	2.25±0.05 d	12.8±0.1 d,e	7.6±0.2 a	3.24	1	2
Vin 6	2.39±0.3 d	2.22±0.02 d	13±0.2 c,d,e	6.7±0.1 b	3.73	0	1
Vin 7	2.49±0.1 c	2.28±0.02 d,e	13.9±0.1 b	5.9±0.2 d	3.52	0	0
Vin 8	2.04±0.4 f,g	1.90± 0.00 b,c	12.6±0.2 d,e	3.7±0.1 h	3.89	1	2
Vin 9	2.07±0.3 f	1.95±0.02 c	12.7±0.1 d,e	3.7±0.3 h	4.18	0	2
Vin 10	2.30±0.5 e	2.22±0,01 d	13.1±0.2 c,d	6.1±0.2 c	3.70	0	1
Vin 11	1.59±0.2 h	1.40±0.05 a	12.9±0.1 c,d,e	5.1±0.2 e,f	3.67	0	1
Vin 12	2.56±0.2 b	2.38±0.05 e	11.9±0.2 e	6.2±0.2 c	3.31	1	2
Vin 13	2.75±0.1 a	2.18±0.05 f	13.4±0.2 c	5.4±0.1 e	3.51	1	2
Vin 14	2.30±0.3 d	1.48±0.02 a	12.0±0.2 e	7.2±0.2 a,b	3.41	1	2

Continued in next page

Continued

Vintage 2013							
Code	**Berry Weight**	**Berry volume**	**Baume**	**Titratable acidity**	**pH**	**Botrytis inf.**	**Seed color.**
Vin 1	2.17±0.3 c	2.03±0.02 f,g	14.1±0.2 d	6.2±0.2 b	3.67	0	0
Vin 2	1.55±0.4 j	1.43± 0.02 a	13.4±0.2 e	5.5±0.3 c	3.67	0	1
Vin 3	1.82±0.3 g	1.73± 0.02 c,d	12.5±0.2 g	4.8±0.1 d	3.63	0	1
Vin 4	2.34±0.3 a	2.30±0.10 h	13.6±0.2 d.e	5.3±0.1 c,d	3.74	0	1
Vin 5	2.23±0.3 b	2.05±0.05 f,g	14.1±0.1 c	7.1±0.3 a	3.54	0	0
Vin 6	1.74±0.2 h	1.60±0.00 b,c	14.1±0.1 b	5.6±0.2 c	3.53	0	0
Vin 7	1.66±0.2 i	1.55±0.05 a,b	13.4±0.2 e	6.3±0.1 b	3.67	0	1
Vin 8	1.79±0.2 g	1.65±0,00 b,c,d	12.1±0.3 h	4.9±0.1 d	3.56	0	2
Vin 9	2.04±0.2 d	1.90±0.10 e,f	13.1±0.2 f	5.2±0.1 d	3.89	0	2
Vin 10	1.88±0.2 f	1.78±0.07 d,e	13.1±0.1 f	6.3±0.2 b	3.56	0	1
Vin 11	1.99±0.1 e	1.90±0.00 e,f	14.2±0.1 a	4.5±0.2 e	3.87	0	0
Vin 12	2.20±0.2 b,c	2.00±0.00 f	12.3±0.1 h	7.1±0.2 a	3.21	0	2
Vin 13	-	-	-	-	-	-	-
Vin 14	2.31±0.2 a	2.18±0.02 g,h	12.6±0.1 g	7.3±0.1 a	3.4	0	2

Vintage 2014							
Code	**Berry Weight**	**Berry volume**	**Baume**	**Titratable acidity**	**pH**	**Botrytis inf.**	**Seed color.**
Vin 1	2.23±0.8 b	1.98±0.02 f	14.5±0.2 a	5.0±0.2 f	3.65	0	0
Vin 2	1.73±0.4 f	1.58±0.02 a,b	12.9±0.1 d	5.1±0.1 f	3.49	0	2
Vin 3	1.96±0.1 d	1.78±0.02 c,d,e	13.3±0.3 c	4.5±0.2 g	3.64	1	1
Vin 4	2.25±0.5 b	2.10±0.02 c,d,e	14±0.2 b	6.2±0.2 d	3.41	0	1
Vin 5	2.53±0.6 a	2.38±0.02 i	11±0.2 f	8.6±0.1 a	3.17	0	2
Vin 6	1.70±0.3 f	1.70±0,10 c,d	13.1±0.3 c,d	7.6±0.2 b	3.43	1	2
Vin 7	1.94±0.2 d	1.80±0.00 d,e	13±0.1 d	6.1±0.1 d	3.43	1	2
Vin 8	1.83±0.6 e	1.68±0.02 b,c	13.1±0.2 c,d	5.8±0.2 e	3.52	2	2
Vin 9	1.77±0.5 e,f	1.55±0.05 a	12.2±0.1 e	5.7±0.1 e	3.42	2	2
Vin 10	1.95±0.3 d	1.83±0.02 e	12.8±0.3 d	6.4±0.3 d	3.4	1	0
Vin 11	2.24±0.1 b	2.10±0.00 g	14±0.2 b	5.0±0.1 f	3.69	0	1
Vin 12	2.24±05 b	2.03±0.02 f,g	12±0.1 e	7.8±0.1 b	3.28	0	2
Vin 13	2.47±0.5 a	2.25±0.05 h	14.0±0.1 b	7.05±0.2 c	3.41	0	1
Vin 14	2.11±0.4 c	1.98±00.5 f	13.8±0.1 b	7.3±0.2 c	3.39	0	1

*Titratable acidity expressed as tartaric acid g/l.

** Botrytis infection: 0 = no infection, 1= < 5% infection, 2 = > 5%infection.

***According to Fredes et al. (2010).

Values with different letters are significantly different ($p<0.05$).

In all cases, differences were reported only among the values of the individual vineyards, depicting the great variations that exist in the different sub regions of the Nemea wine region. The results concerning grape must analysis of Vin 1, Vin 7, Vin 8, Vin 9 and Vin 11 grapes were more or less similar among the three vintages examined unlike those obtained from Vin 2, Vin 4, Vin 10 and Vin 12 which were more influenced by the harvest year. Among vintages no significant differences in grape phenolics were reported, but phenolic compounds of the corresponding wines were significantly increased during 2012 suggesting possible differences in their organoleptic properties. This might be due to differences in grape maturity that affects extraction of phenolic compounds into wine. It has been shown that the extractability of cell walls is increasing with the progress of ripening (Bindon et al., 2014). Moreover, extraction of tannins is also affected by their interactions with soluble polysaccharides present in the grape must (Gil et al., 2012).

The harvest of 2014, took place earlier due to weather conditions as indicated by reduced sugar maturity but also from lower pH values. Insufficiently ripened grapes may produce more astringent and bitter wines due to higher seed proanthocyanidin content, (Romeyer et al., 1985).

4.2.2 Spectrophotometric analyses of phenolic compounds in grape skin and seed extracts.

In Table 4.3, are presented the results of the spectrophotometric analyses of grape skins phenolic compounds. In more detail methyl cellulose tannin assay values ranged from 3.27 to 57.54 mg/l of catechin equivalents, DMAC index from 2.41 to 24.52 mg/l of

catechin equivalents**,** total tannin by acid hydrolysis from 10.98 to 61.62 g/l, Folin-Ciocalteau from 9.39 to 41.11 mg/l of gallic acid equivalents, Adams-Harbertson assay values from 0.90 to 6.69 mg/l, radical scavenging activity (%) ranged from 19.94 to 62.07% and finally DPPH radical-scavenging activity from 0.31 to 0.92 of mM trolox equivalents.

In Table 4.4, are presented the results of the spectrophotometric analyses of grape seed phenolic compounds. DMAC index ranged from 21.84 to 45.11 mg/l of catechin equivalents, total tannin by acid hydrolysis from 24.67 to 130.82 g/l, Folin-Ciocalteau from 33.23 to 254.73 mg/l of gallic acid equivalents, Adams-Harbertson assay values from 5.41 to 17.85 mg/l, radical scavenging activity (%) ranged from 45.67 to 62.01% and finally DPPH radical-scavenging activity from 0.77 to 2.43 mM of trolox equivalents.

In all spectrophotometric analyses of phenolic compounds, grape seed extracts presented higher values than the skin extracts in agreement with literature (Obreque-Slier et al., 2010; Bonada et al., 2015; Casassa et al., 2015; Chira et al., 2015). Differences among vintages were reported. Vintage 2013 was characterized by lower values of both skin and seed phenolic compounds by all methods applied (Table 4.3 and 4.4). These differences were greater for the methods: methyl cellulose, acid hydrolysis and Folin-Ciocalteau where the values of 2012 were in most cases double than 2013. As earlier presented (Table 4.1) the growing season of 2012 was characterized by elevated temperatures from flowering until harvest in comparison to 2013. In 2012, heavy rainfall occurred during harvest resulting to heavy botrytis load, and reduced grape quality. Unlike 2012 growing season, 2013 was a cool season with low average temperatures during vegetative growth, slow maturation, lack of rainfall

during harvest and minimum botrytis infection. Weather conditions and viticultural parameters are known parameters affecting phenolic composition and in literature, great variations have been reported (Lorrain et al., 2011) among the different experimentation years. It is generally reported that for weather conditions similar to 2012, greater grape phenolic content is expected more likely due to increased water supply (Kennedy et al., 2000; Lorrain et al., 2011; Zarrouk et al., 2012), delayed maturity (Kennedy et al., 2000; Chira et al., 2011) and reduced ambient temperature (Spayd et al., 2002).

Table 4.3: Spectrophotometric analyses of skin extract phenolic compounds (per gr fresh weight) for vintage 2012 and 2013 (next page).

Vintage 2012														
Code	**Methyl cell**[1]		**DMAC**[2]		**Acid hydr.** [3]		**Folin**[4]		**Haberson**[5]		**DA (%)**[6]		**DPPH**[7]	
Vin 1	24.16±0.08	g	9.75±0.22	c	15.57±0.93	a	14.59±0.12	d	4.34±0.13	f	54.87±0.21	a,b	0.50±0.02	f
Vin 2	24.39±0.41	g	8.42±0.47	b	38.07±1.82	g	14.32±0.09	c	1.99±0.01	b,c	54.02±0.41	a	0.36±0.03	b
Vin 3	24.34±0.66	g	15.06±0.10	e	37.14±0.58	g	22.43±0.06	h	6.69±0.38	h	61.42±1.17	e	0.52±0.01	g
Vin 4	57.54±0.07	j	7.16±0.01	a	27.68±2.90	d,e	10.46±0.07	b	2.24±0.40	c,d	55.89±1.34	a,b,c	0.39±0.03	c
Vin 5	13.80±0.14	f	6.85±0.44	a	21.24±0.24	b	10.38±0.02	b	2.07±0.10	b,c	58.80±1.18	c,d,e	0.31±0.01	a
Vin 6	3.27±0.04	a	8.09±0.02	b	23.99±0.28	c	6.78±0.05	a	0.90±0.03	a	58.95±1.14	c,d,e	0.29±0.01	a
Vin 7	13.82±0.08	f	16.22±0.19	f	47.07±0.44	h	26.64±0.13	k	3.70±0.95	e	58.97±0.47	c,d,e	0.55±0.001	h
Vin 8	13.40±0.04	e	16.49±0.29	f	45.21±2.62	h	24.34±0.16	j	2.60±0.62	d	59.79±0.30	d,e	0.92±0.02	l
Vin 9	3.58±0.12	b	15.90±0.33	f	35.99±0.95	f,g	23.08±0.14	i	1.68±0.01	b	57.88±0.22	b,c,d	0.82±0.03	k
Vin 10	46.96±0.80	h	24.52±1.08	h	54.14±1.34	i	41.11±0.11	l	6.06±0.39	i	61.27±0.18	e	0.81±0.02	k
Vin 11	13.51±0.66	e	9.69±0.23	c	25.54±1.97	d	15.71±0.13	e	4.12±0.12	e,f	55.48±1.16	a,b	0.42±0.01	d
Vin 12	55.37±0.14	i	13.61±1.32	d	24.24±0.09	c,d	21.32±0.47	g	2.69±0.32	d	62.07±4.3	e	0.47±0.06	e
Vin 13	4.42±0.19	c	7.17±0.12	a	61.62±0.81	j	20.44±0.11	f	2.19±0.11	b,c,d	56.57±0.17	a,b,c	0.41±0.02	c,d
Vin 14	9.48±0.09	d	19.17±0.28	g	36.61±2.28	f,g	20.67±0.07	f	4.93±0.01	g	54.92±0.06	a,b	0.58±0.01	i

[1] tannin concentration determined by the methyl cellulose method expressed as mg/l of epicatechin equivalents.

[2] tannin concentration determined by the DMAC index expressed as g/l.

[3] tannin concentration determined by acid hydrolysis method expressed as g/l.

[4] tannin concentration determined by the Folin-Ciocalteau assay expressed as g/l gallic acid equivalents.

[5] tannin concentration determined by the Adams-Harbertson assay expressed as mg/l of epicatechin equivalents.

[6] tannin concentration determined by the DPPH method expressed as mM trolox.

[7] radical scavenging activity (%).

Values with different letters are significantly different ($p<0.05$). Values are the means of triplicate determinations +/- standard deviation.

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Vintage 2013														
Code	**Methyl cell**[1]		**DMAC**[2]		**Acid hydr.** [3]		**Folin**[4]		**Haberson**[5]		**DA (%)**[6]		**DPPH**[7]	
Vin 1	10.47±0.81	c,d	3.64±0.15	e,f	10.98±1.42	a	10.94±0.19	b	1.12±0.09	a,b	23.72±1.38	c,d	0.35±0.012	b,c
Vin 2	12.77±0.59	d,e	3.99±0.04	f,g	30.69±3.22	b,c	18.65±0.04	h	1.98±0.11	f	31.07±0.06	g	0.51±0,01	g
Vin 3	8.80±1.26	a,b,c	3.41±0.05	d,e	18.76±6.80	a,b	10.40±0.08	b	1.28±0.13	b,c	20.94±0.59	a	0.32±0.05	a
Vin 4	6.55±0.13	a	3.85±0.08	f,g	22.22±5.05	a,b,c	13.14±0.29	d	2.11±0.10	f,g	23.81±0.41	c,d	0.35±0.04	b,c
Vin 5	9.45±2.32	b,c	3.15±0.02	c,d	27.05±8.58	a,b,c	11.74±1.15	b	1.64±0.05	d	20.66±0.57	a	0.38±0.06	d
Vin 6	10.06±3.16	c,d	3.10±0.24	c,d	30.69±3.99	b,c	13.20±0.31	d	1.40±0.07	c	23.34±0.64	b,c	0.37±0.06	c,d
Vin 7	13.37±0.22	e	4.08±0.14	g	38.50±7.24	c,d	17.17±0.26	g	2.18±0.08	g	23.93±0.19	c,d	0.48±0.02	f
Vin 8	10.95±0.29	c,d,e	4.71±0.42	h	17.54±0.73	a,b	13.02±0.26	d	2.11±0.01	f,g	28.12±0.56	f	0.34±0.04	a,b
Vin 9	9.56±0.35	c	2.79±0.03	b,c	17.83±1.25	a,b	13.34±0.20	d	1.81±0.06	e	21.30±1.37	a,b	0.42±0.02	e
Vin 10	10.79±0.79	c,d,e	3.98±0.21	f,g	29.99±0.79	b,c	15.13±0.31	e	2.26±0.02	g	26.87±0.38	e,f	0.43±0.04	e
Vin 11	10.67±0.16	c,d,e	2.52±0.17	a,b	35.77±8.81	c,d	16.37±0.51	f	1.79±0.03	d,e	20.23±0.98	a	0.51±0.00	g
Vin 12	8.60±1.30	a,b,c	3.76±0.02	e,f,g	49.65±8.10	d	16.49±0.27	f	2.47±0.03	h	25.58±2.59	d,e	0.50±0.03	g
Vin 13	-		-		-		-		-		-		-	
Vin 14	6.75±0.28	a,b	2.41±0.12	a	26.84±4.65	a,b,c	9.39±0.15	a	1.10±0.03	a	19.94±0.85	a	0.32±0.23	a

[1] tannin concentration determined by the methyl cellulose method expressed as mg/l of epicatechin equivalents.

[2] tannin concentration determined by the DMAC index expressed as g/l.

[3] tannin concentration determined by acid hydrolysis method expressed as g/l.

[4] tannin concentration determined by the Folin-Ciocalteau assay expressed as g/l gallic acid equivalents.

[5] tannin concentration determined by the Adams-Harbertson assay expressed as mg/l of epicatechin equivalents.

[6] tannin concentration determined by the DPPH method expressed as mM trolox.

[7] radical scavenging activity (%).

Values with different letters are significantly different ($p<0.05$). Values are the means of triplicate determinations +/- standard deviation.

Table 4.4: Spectrophotometric analyses of seed extracts phenolic compounds (per gr fresh weight) for vintage 2012 and 2013 (next page).

Vintage 2012												
Code	**DMAC**[1]		**Acid hydr.** [2]		**Folin**[3]		**Haberson**[4]		**DA (%)**[5]		**DPPH**[6]	
Vin 1	28.56±0.60	c,d	85.69±2.21	c	159.25±1.62	a	8.98±0.06	c	58.96±0.63	a,b,c	1.83±0.02	a
Vin 2	26.19±0.48	b,c	98.78±4.21	d	193.87±2.56	c,d	9.58±0.17	d	60.09±0.90	a,b,c	2.15±0.01	c,d
Vin 3	31.43±0.17	e,f,g	106.55±1.56	d	254.73±4.90	i	17.85±0.01	i	59.14±0.71	a,b,c	0.23±0.01	f
Vin 4	30.84±0.13	d,e,f,g	92.14±1.78	d	214.61±10.11	e	9.81±0.21	d,e,f	57.85±0.24	a,b,c	2.05±0.02	b,c
Vin 5	32.86±0.48	e,f,g	88.80±1.65	c	178.54±0.33	b	8.48±0.21	b	58.81±1.19	a,b,c	2.03±0.01	b
Vin 6	29.09±0.56	c,d,e	114.39±4.32	e	207.99±4.28	e	9.10±0.05	c	59.50±0.03	a,b,c	2.10±0.01	b,c,d
Vin 7	23.59±0.07	a,b	85.82±1.56	c	210.66±1.47	e	9.87±0.13	f	58.97±0.47	a,b,c	2.05±0.02	b,c
Vin 8	34.51±0.06	g	130.82±2.54	f	198.58±3.75	c,d	11.77±0.03	h	61.43±1.16	b.c	2.43±0.01	g
Vin 9	33.56±0.02	f,g	98.88±3.82	d	231.72±0.79	f,g	12.82±0.35	h	57.88±0.22	a	2.15±0.01	c,d
Vin 10	45.11±1.2.	c,d,e,f	68.65±3.65	a,b	229.21±1.12	f	9.69±0.04	e	61.27±0.17	a,b,c	2.35±0.02	f,g
Vin 11	33.04±0.08	d,e,f,g	106.55±3.52	e	234.15±0.71	g	10.98±0.12	g	59.22±0.13	a,b,c	2.18±0.01	d,e
Vin 12	21.84±0.78	a	76.87±2.36	b	192.07±1.22	c	9.86±0.24	e,f	62.01±4.31	c	2.07±0.01	b,c
Vin 13	27.32±0.27	b,c,d	40.44±1.99	a	244.85±2.59	h	8.43±0.03	b	58.76±0.03	a,b,c	2.26±0.02	e,f
Vin 14	31.07±0.07	d,e,f,g	59.04±4.82	a	195.82±4.96	d	5.81±0.01	a	58.76±0.002	a,b,c	2.20±0.02	d,e

[1] tannin concentration determined by the DMAC index expressed as g/l.

[2] tannin concentration determined by acid hydrolysis method expressed as g/l.

[3] tannin concentration determined by the Folin-Ciocalteau assay expressed as g/l gallic acid equivalents.

[4] tannin concentration determined by the Adams-Harbertson assay expressed as mg/l of epicatechin equivalents.

[5] tannin concentration determined by the DPPH method expressed as mM trolox.

[6] radical scavenging activity (%).* expressed as mg/l of catechin equivalents.

Values with different letters are significantly different ($p<0.05$). Values are the means of triplicate determinations +/- standard deviation.

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Vintage 2013												
Code	**DMAC**[1]		**Acid hydr.** [2]		**Folin**[3]		**Haberson**[4]		**DA (%)**[5]		**DPPH**[6]	
Vin 1	36.06±1.2	g,h	30.47±1.22	c	44.08±1.83	e,f	7.42±0.82	c	56.22±1.2	h.i	1.00±0.02	d,e
Vin 2	31.56±0.84	b,c,d,e	31.87±2.33	c,d	43.50±2.22	e,f	10.84±1.12	e	52.15±1.66	d,e,f	1.06±0.02	f,g
Vin 3	31.18±2.10	b	32.74±3.21	c,d,e	40.59±2.86	c,d	7.41±0.36	c	59.09±2.10	i,j	1.00±0.01	d,e
Vin 4	31.37±1.44	b,c,d	28.46±1.69	b	37.18±1.89	b	5.88±0.66	a,b	55.06±1.55	f,g,h	1.02±0.03	d,e
Vin 5	36.16±1.20	h	35.80±1.88	f	42.50±3.10	d,e	5.97±0.14	a,b	55.92±1.64	g,h	1.09±0.02	g
Vin 6	35.40±0.80	g	31.68±2.36	c,d	43.75±2.89	e,f	7.59±0.26	c	60.99±0.65	j	1.04±0.02	e,f
Vin 7	30.97±1.20	b	29.43±1.6	b,c	40.44±2.66	c,d	6.95±0.12	b,c	49.92±1.20	c,d	0.94±0.01	c
Vin 8	31.29±0.82	b,c	31.50±0.98	c,d	40.39±1.20	c,d	8.82±0.66	d	53.14±2.10	e,f,g	0.99±0.01	d
Vin 9	32.14±0.64	e	25.63±3.65	a	37.04±1.66	b	5.41±0.44	a	45.67±1.36	a	0.83±0.02	b
Vin 10	33.42±1.20	f	28.12±2.36	b	39.84±1.56	c	5.98±0.10	a,b	51.24±1.66	c,d,e	0.98±0.01	c
Vin 11	31.99±2.20	c,d,e	33.32±1.36	d,e	44.97±3.32	f	10.04±0.32	e	52.64±2.24	d,e,f	1.02±0.02	d,e
Vin 12	32.08±1.20	d,e	32.67±1.89	c,d,e	40.71±1.66	c,d	7.82±0.12	c,d	48.94±2.20	b,c	0.94±0.01	c
Vin 13	-		-		-		-		-		-	
Vin 14	26.86±0.88	a	24.67±1.68	a	33.23±2.66	a	5.91±0.31	a,b	46.23±1.22	a,b	0.77±0.02	a

[1] tannin concentration determined by the DMAC index expressed as g/l.

[2] tannin concentration determined by acid hydrolysis method expressed as g/l.

[3] tannin concentration determined by the Folin-Ciocalteau assay expressed as g/l gallic acid equivalents.

[4] tannin concentration determined by the Adams-Harbertson assay expressed as mg/l of epicatechin equivalents.

[5] tannin concentration determined by the DPPH method expressed as mM trolox.

[6] radical scavenging activity (%).* expressed as mg/l of catechin equivalents.

Values with different letters are significantly different ($p<0.05$). Values are the means of triplicate determinations +/- standard deviation.

4.2.3 Anthocyanin analysis by High Performance Liquid Chromatography (HPLC).

In Table 4.5 are presented the results of skin anthocyanin concentration performed by HPLC. A vintage effect was observed but not in all anthocyanins: delphinidin, cyanidin, petunidin and peonidin presented similar values among vintages while malvidin and its derivatives were more influenced. The highest values were observed during vintage 2014 and the lowest during 2012. Ambient temperature was similar between vintages 2013 and 2014 while in 2012 it was elevated. In contrast to literature, where elevated temperature could decrease skin anthocyanin content (Pereira et al., 2006; Cortell et al., 2007; Tarara et al., 2008), we reported a converse effect only on malvidin and its derivatives.

Irrigation is an important parameter affecting skin anthocyanin content and a positive effect of water deficit is reported by many authors (Castellarin et al., 2007; Chacon et al., 2009; Bucchetti et al., 2011; Kyraleou et al., 2015). The concentration of the hormone abscisic acid (ABA) might play a critical role in regulating the acceleration of berry pigmentation under water deficit. ABA concentration in the berry increases remarkably at veraison (Owen et al., 2009) and several studies indicated that ABA stimulates the synthesis of anthocyanins in grapevine by promoting the expression of key biosynthetic genes (Jeong et al., 2004; Gambetta et al., 2010). Water deficit increases ABA concentration and as a result water deficit could hasten the beginning of ripening extending the grape ripening period. In contrast, in our experiment in 2014 when we reported the highest malvidin and its derivatives concentration, water supply was higher (before and after veraison) opposite to 2013 when water supply was restricted during vine growth and 2012 when water deficit occurred before veraison (Table 4.1).

It is known that when severe water limitation occurs early in the season, the growth pattern of productive vines is modified and morpho-functional and biochemical adaptive mechanisms are used by vines to withstand stress. Even though, the effects of drought on development and functions of *Vitis vinifera* are well documented, little is known about recovery of physiological processes after re-watering and especially their influences on vine yield and grape composition. Some cultivars seem to perform better in recovering after water stress (Pou et al., 2012; Palliotti et al., 2014) suggesting that genotype plays a critical role. Palliotti et al. (2014), investigated the effect of pre-veraison water stress and recovery after water supply on Sangiovese and Montepulciano vines. Montepulciano was negatively influenced, as total anthocyanins and phenolics decreased under stress, suggesting that its ideal environment requires well-watered conditions to achieve optimum fruit chemical composition, whereas a pre-veraison water deficit was beneficial in Sangiovese. It is possible that Agiorgitiko belongs to the category of cultivars that require moderate water supply through ripening to obtain optimum fruit composition. However, since many parameters such as weather conditions, viticultural treatments, vinification conditions influence the final wine anthocyanin composition the effect of water supply at this phenological stage and vine`s performance and recovery is often neglected.

Table 4.5: Anthocyanin concentration measured by HPLC (mg anthocyanins/g grape fresh weight) of grape skins for vintage 2012, 2013 and 2014 (next pages).

Vintage 2012														
Code	**Dlp**		**Cyan**		**Pt**		**Pn**		**Mlv**		**Mlv Ac**		**Mlv Coum**	
Vin 1	0.48±0.02	g	0.15±0.01	b,c	0.60±0.02		0.53±0.02	e	7.74±0.11	f	0.27±0.01	d	2.17±0.02	e
Vin 2	0.19±0.03	b	0.11±0.02	a	0.26±0.02	b	0.36±0.02	b	2.61±0.22	a	0.19±0.02	b	1.00±0.06	a
Vin 3	0.15±0.01	a	0.10±0.01	a	0.20±0.01	a	0.30±0.02	a	2.65±0.22	a	0.23±0.01	c	1.51±0.05	b,c
Vin 4	0.23±0.01	c	*nd*	-	0.31±0.06	c	0.52±0.02	e	4.14±0.13	b	0.25±0.02	c	1.64±0.08	c
Vin 5	0.65±0.05	i	0.18±0.01	d	0.76±0.06	i	0.64±0.03	g	6.06±0.23	e	0.19±0.02	b	1.36±0.10	b
Vin 6	0.21±0.02	c	0.12±0.01	a,b	0.28±0.03	b	0.46±0.02	c,d	4.10±0.22	b	0.24±0.01	c	1.36±0.02	b
Vin 7	0.39±0.02	e	0.15±0.01	b,c	0.49±0.03	f	0.66±0.01	g	6.11±0.24	e	0.19±0.01	b	1.84±0.06	d
Vin 8	0.41±0.01	f	0.13±0.01	b	0.49±0.02	f	0.45±0.01	c,d	4.79±0.18	b,c	0.17±0.01	b	2.12±0.04	e
Vin 9	0.22±0.04	c	*nd*	-	0.35±0.03	d	0.78±0.01	h	4.12±0.14	b	0.23±0.02	c	1.45±0.06	b,c
Vin 10	0.22±0.03	c	0.11±0.01	a	0.30±0.03	b,c	0.43±0.03	c	4.45±0.08	b	0.22±0.02	b,c	1.81±0.08	d
Vin 11	0.39±0.02	e	0.13±0.01	b	0.48±0.02	f	0.49±0.05	d	5.02±0.80	c	0.21±0.01	b,c	2.23±0.04	f
Vin 12	0.56±0.04	h	0.18±0.01	d	0.64±0.02	h	0.68±0.02	h,i	5.77±0.26	d	0.15±0.01	a	1.60±0.12	c
Vin 13	0.32±0.03	d	0.14±0.01	b	0.40±0.01	e	0.58±0.02	f	4.24±0.32	b	0.18±0.01	b	1.60±0.05	c
Vin 14	0.48±0.06	g	0.19±0.01	d	0.58±0.6	g	0.87±0.03	j	5.02±0.20	c	0.17±0.01	b	1.36±0.03	b

Values are the means of triplicate determinations +/- standard deviation.

Values with different letters are significantly different ($p<0.05$).

Abbreviations: *Dlp, Cyan, Pt, Pn* and *Mlv* stand for 3-O-glucosides of delphinidin, cyaniding, petunidin, peonidin and malvidin respectively. *Mlv Ac:* malvidin-3-O-glucose acetate, *Mlv Coum:* malvidin-3-O-glucose coumarate.

nd: not detected.

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Vintage 2013														
Code	**Dlp**		**Cyan**		**Pt**		**Pn**		**Mlv**		**Mlv Ac**		**Mlv Coum**	
Vin 1	0.34±0.01	g	0.15±0.01	f	0.43±0.05	e,f	0.81±0.02	h	5.51±0.11	e	0.42±0.01	d	2.14±0.04	d
Vin 2	0.40±0.06	h	0.12±0.01	d	0.50±0.08	g	0.51±0.09	d,e	6.08±0.09	f	0.63±0.05	h	2.97±0.01	f
Vin 3	0.26±0.06	d	0.10±0.01	b	0.34±0.07	c	0.44±0.01	c	4.65±0.14	c	0.48±0.01	f	2.23±0.06	e
Vin 4	0.19±0.01	a	0.10±0.01	b	0.26±0.01	a	0.35±0.01	b	4.00±0.01	b	0.41±0.01	d	2.16±0.01	d
Vin 5	0.57±0.01	k	0.23±0.01	i	0.69±0.01	i	1.26±0.02	j	7.28±0.12	h	0.39±0.01	c	1.90±0.03	b
Vin 6	0.30±0.06	e	0.11±0.01	c	0.38±0.01	d	0.51±0.01	d,e	5.21±0.04	d	0.35±0.01	b	1.95±0.02	b
Vin 7	0.34±0.02	f,g	0.14±0.01	e	0.42±0.01	e	0.55±0.01	f	6.48±0.03	g	0.62±0.01	h	3.11±0.09	g
Vin 8	0.22±0.01	c	0.08±0.01	a	0.29±0.01	b	0.24±0.01	a	3.49±0.02	a	0.37±0.01	b	1.95±0.08	b
Vin 9	0.21±0.01	b	0.12±0.01	c	0.30±0.06	b	0.51±0.03	e	4.52±0.09	c	0.48±0.02	f	2.16±0.06	d
Vin 10	0.33±0.03	f	0.11±0.01	c	0.43±0.04	e,f	0.49±0.01	d	6.51±0.11	g	0.58±0.01	g	2.95±0.05	f
Vin 11	0.41±0.01	i	0.21±0.01	h	0.52±0.01	h	1.31±0.01	k	5.12±0.01	d	0.37±0.01	b	2.04±0.01	c
Vin 12	0.72±0.04	l	0.23±0.01	j	0.83±0.01	j	1.04±0.01	i	7.90±0.02	i	0.46±0.01	e	2.17±0.01	d
Vin 13	-		-		-		-		-		-		-	
Vin 14	0.40±0.01	h,i	0.18±0.01	g	0.44±0.04	f	0.75±0.02	g	3.97±0.07	b	0.24±0.01	a	1.08±0.03	a

Values are the means of triplicate determinations +/- standard deviation.

Values with different letters are significantly different ($p<0.05$).

Abbreviations: *Dlp, Cyan, Pt, Pn* and *Mlv* stand for 3-O-glucosides of delphinidin, cyaniding, petunidin, peonidin and malvidin respectively. *Mlv Ac:* malvidin-3-O-glucose acetate, *Mlv Coum:* malvidin-3-O-glucose coumarate.

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Vintage 2014														
Code	**Dlp**		**Cyan**		**Pt**		**Pn**		**Mlv**		**Mlv Ac**		**Mlv Coum**	
Vin 1	0.90±0.01	f	0.13±0.01	b	0.95±0.02	h	0.99±0.01	f	9.68±1.22	d	0.28±0.06	b	2.27±0.02	g
Vin 2	0.28±0.01	a	0.05±0.01	a	0.40±0.01	a	0.75±0.01	c	5.85±1.64	a	0.28±0.06	b	1.94±0.02	d
Vin 3	1.15±0.01	g	0.22±0.01	c	1.13±0.02	i	1.27±0.02	h	9.97±1.66	d	0.24±0.01	b	1.75±0.01	c
Vin 4	-		-		-		-		-		-		-	
Vin 5	0.35±0.01	b	0.06±0.01	a	0.47±0.01	b	0.60±0.01	a,b	6.29±1.88	a,b	0.39±0.01	c	2.44±0.01	h
Vin 6	0.76±0.01	e	0.14±0.01	b	0.89±0.02	g	1.34±0.02	i	10.12±0.86	d	0.56±0.08	e	3.40±0.06	j
Vin 7	0.51±0.01	c	0.03±0.01	a	0.61±0.02	d	0.51±0.03	a	6.84±1.00	a,b	0.55±0.15	e	2.84±0.04	i
Vin 8	0.53±0.01	c	0.08±0.0	a,b	0.60±0.02	d	0.85±0.03	d	7.68±1.32	c	0.46±0.02	d	2.31±0.02	g
Vin 9	0.46±0.01	b,c	0.04±0.01	a	0.53±0.02	c	0.50±0.02	a	7.19±1.10	c	0.43±0.03	d	2.13±0.02	f
Vin 10	0.33±0.01	b	0.05±0.01	a	0.42±0.02	a	0.79±0.01	c	5.59±1.88	a	0.37±0.01	c	2.02±0.01	e
Vin 11	0.69±0.01	e	0.12±0.0	b	0.76±0.02	f	0.69±0.01	b	7.03±0.98	c	0.15±0.02	a	1.51±0.01	a
Vin 12	058±0.12	d	0.11±0.01	b	0.70±0.01	e	1.15±0.03	g	6.90±1.66	a,b	0.28±0.01	b	2.18±0.02	f
Vin 13	-		-		-		-		-		-		-	
Vin 14	0.38±0.01	b	0.12±0.01	b	0.48±0.01	b	0.90±0.01	e	5.26±2.006	a	0.16±0.02	a	1.60±0.01	b

Values are the means of triplicate determinations +/- standard deviation.

Values with different letters are significantly different ($p<0.05$).

Abbreviations: *Dlp, Cyan, Pt, Pn* and *Mlv* stand for 3-O-glucosides of delphinidin, cyaniding, petunidin, peonidin and malvidin respectively. *Mlv Ac:* malvidin-3-O-glucose acetate, *Mlv Coum:* malvidin-3-O-glucose coumarate.

4.2.4 Spectrophotometric analyses of grape skin phenols and anthocyanins.

In Table 4.6 are presented the results of the spectrophotometric determination of total anthocyanins in red grape berries according to Illand et al., (2000). The anthocyanins expressed as per mg/berry ranged from 0.94 (Vin 14, 2013) to 3.11 (Vin 12, 2012), anthocyanins expressed as per berry weight (g) ranged from 0.43 (Vin 14, 2013) to 1.28 (Vin 12, 2012), phenols ranged from 1.66 (Vin 7, 2013) to 3.82 au (absorbance units at 280 nm) (Vin 7, 2012) and finally phenols expressed per berry weight (gr) ranged from 0.75 (Vin 4, 2013) to 1.74 (Vin 14, 2012). The results of anthocyanin content were similar among vintages and vineyards and differences were reported only in the phenolic content of the grape berries. In 2012, phenolic content (au) was higher against the other years of the experiment among which no differences were reported. However, when the phenolic content was expressed as per berry weight these differences were not evident. In all measurements (anthocyanin and phenolics) all highest values were reported during vintage 2012 and the lowest in 2013.

Berry volume and weight did not affect the anthocyanin and phenolic content of the grapes. As seen in Tables 4.2 and 4.6, the highest and lowest values were not observed at vineyard samples with the proportionally highest or lowest berry weight and/or volume. Bindon et al. (2008), reported a poor correlation between berry size and phenolic and anthocyanin concentration, applying the `Illand` method, but a significant positive correlation was found between berry size and anthocyanins per berry. Overall, the results obtained in this study showed a weak relation between secondary metabolite concentration and berry size unlike the general belief that smaller berries are more concentrated and thus positively related to wine quality.

Table 4.6: Spectrophotometric determination of total anthocyanins in red grape berries based on the methods described by Illand et al. (2000).

Vintage 2012								
Code	**Anth. mg/berry**		**Anth./ per gr berry**		**Phenols (au)**		**Phenols /gr berry**	
Vin 1	1.97±0.06	c	0.95±0.03	c,d	3.35±0.02	e	1.61±0.01	d,e
Vin 2	1.37±0.05	a	0.69±0.03	a,b	2.30±0.02	a	1.15±0.01	a
Vin 3	1.91±0.03	c	0.82±0.01	b,c	3.33±0.02	e	1.43±0.01	c
Vin 4	1.33±0.01	a	0.74±0.03	a,b	2.62±0.01	b	1.46±0.01	c
Vin 5	2.12±0.01	d	0.84±0.01	b,c	2.99±0.01	d	1.19±0.01	a,b
Vin 6	1.61±005	b	0.68±0.04	a	2.61±0.03	b	1.10±0.01	a
Vin 7	2.71±0.04	f	1.14±0.02	e	3.82±0.06	g	1.60±0.03	d,e
Vin 8	1.46±0.05	a,b	0.71±0.02	b	2.67±0.03	b	1.29±0.01	b
Vin 9	1.49±0.06	a,b	0.76±0.02	b	2.76±0.02	c	1.40±0.01	c
Vin 10	1.34±0.05	a	0.64±0.03	a	2.51±0.03	a,b	1.21±0.01	a,b
Vin 11	1.48±0.02	a,b	0.90±0.03	c	2.58±0.01	b	1.56±0.01	d
Vin 12	3.11±0.01	g	1.28±0.09	f	3.73±0.01	f	1.53±0.01	c,d
Vin 13	2.51±0.05	e	0.97±0.01	c,d	3.74±0.05	f	1.46±0.02	c
Vin 14	1.98±0.01	c	1.25±0.01	f	2.77±0.02	c	1.74±0.01	f

Vintage 2013								
Code	**Anth. mg/berry**		**Anth./ per gr berry**		**Phenols (au)**		**Phenols /gr berry**	
Vin 1	2.04±0.10	f	0.87±0.05	e	3.08±0.07	j	1.32±0.03	c
Vin 2	1.68±0.02	e	0.96±0.01	f	2.45±0.09	h	1.40±0.05	d
Vin 3	1.50±0.07	d	0.83±0.04	d,e	2.95±0.08	i	1.63±0.05	f
Vin 4	1.23±0.09	b,c	0.54±0.04	b	1.73±0.06	b	0.75±0.03	a
Vin 5	1.69±0.03	e	0.78±0.02	d	3.25±0.11	k	1.52±0.05	e
Vin 6	1.63±0.03	e	0.91±0.01	e,f	2.09±0.04	d	1.16±0.02	a,b
Vin 7	1.19±0.04	b	0.74±0.02	d	1.66±0.01	a	1.03±0.06	a,b
Vin 8	1.60±0.01	e	0.78±0.01	d	2.36±0.06	g	1.14±0.03	b
Vin 9	1.45±0.01	d	0.75±0.01	d	2.18±0.07	e	1.13±0.04	b
Vin 10	1.56±0.03	d,e	0.87±0.02	e	2.48±0.08	h	1.39±0.04	d
Vin 11	1.36±0.16	c	0.68±0.08	c,d	2.04±0.20	c	1.03±0.10	a,b
Vin 12	1.38±0.07	c	0.62±0.03	c	2.97±0.09	i	1.33±0.04	c
Vin 13	-		-		-		-	
Vin 14	0.94±0.06	a	0.43±0.03	a	2.24±0.16	f	1.03±0.07	a,b

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Vintage 2014								
Code	**Anth. mg/berry**		**Anth./ per gr berry**		**Phenols (au)**		**Phenols /gr berry**	
Vin 1	1.65±0.15	e	0.82±0.07	d	2.26±0.14	b	1.12±0.07	c
Vin 2	1.56±0.14	d	0.93±0.08	e	2.07±0.09	a	1.23±0.05	d
Vin 3	1.41±0.16	c	0.76±0.09	c	2.29±0.02	b	1.24±0.01	d
Vin 4	1.54±0.08	d	0.67±0.06	b	2.42±0.04	d	1.06±0.04	b
Vin 5	1.81±0.13	f	0.79±0.06	c	2.14±0.13	a	0.93±0.06	a
Vin 6	1.90±0.11	g	1.09±0.06	f	2.29±0.05	b	1.31±0.03	e,f
Vin 7	1.96±0.25	h	1.06±0.13	f	2.19±0.30	a	1.18±0.16	c
Vin 8	1.14±0.17	a	0.67±0.10	b	2.10±0.29	a	1.23±0.17	d
Vin 9	1.26±0.14	b	0.65±0.07	b	2.47±0.07	e	1.28±0.04	e
Vin 10	1.87±0.34	f,g	1.11±0.20	f,g	2.30±0.11	b,c	1.37±0.06	g
Vin 11	1.81±0.07	f	0.86±0.03	d,e	2.56±0.11	f	1.22±0.05	d
Vin 12	1.23±0.08	b	0.59±0.04	a	2.13±0.06	a	1.02±0.03	b
Vin 13	-		-		-		-	
Vin 14	1.83±0.24	f,g	0.94±0.12	e	2.23±0.34	b	1.14±0.17	c

Values represent means of triplicate determinations ± standard deviation.

Values with different letters are significantly different ($p<0.05$).

Finally comparing our results with relevant results obtained for several international grape varieties we reported higher values than Syrah (Bindon et al., 2008); Merlot and Cabernet Sauvignon from various growing regions of Australia (1996-2003) from Australian Wine research Institute (AWRI). Moreover, as regards Agiorgitiko in comparison with other indigenous Greek varieties, is a variety rich in anthocyanins with higher values than most of the Greek varietals (Kallithraka et al., 2005; Kallithraka et al., 2009; Kyraleou et al., 2014). Compared with international varieties grown in Greece, Agiorgitiko presented similar values to Syrah, Cabernet Sauvignon and Merlot (Kallithraka et al., 2009; Koundouras et al., 2009; Kyraleou et al., 2016).

The method developed by Glories is one of the most widely used to determine grape phenolic compounds and in Table 4.7 are presented the results from the three years experiment. Total anthocyanins (mg/l) ranged from 0.21 (Vin 9. 2012) to 0.60 (Vin

14,2014), anthocyanin extractability (AE%) from 17.12 (Vin 5, 2012) to 51.97% (Vin 8, 2013), total phenolics (OD 280) from 24.19 (Vin 5, 2014) to 36.87 (Vin 1, 2014), the contribution of seed tannins to the total phenol content (MP%) ranged from 60.11 (Vin 10, 2014) to 84.61% (Vin 9, 2012), skin tannin concentration (Dpell) ranged from 4.94 (Vin 9, 2012) to 13.43 mg/l (Vin 6, 2014), seed tannin concentration (Dpep) from 17.05 (Vin 7, 2014) to 28.57 mg/l (Vin 1, 2012). Finally the contribution (%) of skin and seed tannins to the total tannin content ranged from 15.38 and 84.61% (Vin 9, 2012) to 42.29 and 57.70% (Vin 14, 2014), respectively. Comparing vintages, overall the results for anthocyanin content (mg/l), total phenolics and anthocyanin extractability (AE %) were similar. Differences were observed on the skin and seed phenolic content and their proportion, where in 2012 seed phenolic content and contribution was elevated in comparison with the other vintages. As previously discussed in 2012, even though sugar maturity was reached, phenolic maturity was not; resulting in unbalanced wines compared to vintage 2013 and 2014. In general, total tannin content of seeds shows an increasing trend from bunch closure to veraison and thereafter decreases from veraison to fruit maturity as reported by Romeyer et al., (1985); Katalinic and Males, (1997); Kennedy et al., (2000); Rabot et al., (2017). Moreover, the lower the EA% and MP% values, the riper the berry (Villango et al., 2015). Therefore, skin and seed tannin content and their contribution to the total tannin content of the berry might be a good indicator of grape phenolic maturity but alone these measurements are insufficient to establish optimal harvest date, since it is not providing adequate grape quality characteristics (e.g. aromatic compounds. color intensity etc.).

Table 4.7: Grape phenolic parameters determined by the method developed by Glories and Augustin (1993).

Vintage 2012																
Code	**T. Anth. (gr/lt)**		**AE (%)**		**OD280 (au)**		**MP (%)**		**Dpell (mgr/lt)**		**Dpell (%)**		**Dpep (mgr/lt)**		**Dpep (%)**	
Vin 1	0.31±0.01	a	44.74±1.22	a,b,c	35.42±1.12	k	80.67±0.05	f	6.85±0.42	b	19.33±0.42	b	28.57±0.23	e	80.67±0.05	g
Vin 2	0.29±0.01	a	35.75±2.36	b,c,d	34.59±1.10	h	78.10±0.20	e	7.57±0.80	c	21.89±0.08	c	27.01±0.18	c,d	78.10±0.20	f
Vin 3	0.25±0.01	b	22.44±3.60	b,c,d	32.42±0.80	d	75.58±0.24	d	7.91±1.20	c	24.41±0.84	d	24.50±0.22	b,c	75.58±0.24	d,e
Vin 4	0.34±0.02	a	31.34±0.10	a,b	30.90±1.20	a	69.54±0.66	b	9.41±0.09	e	30.45±0.66	f	21.48±0.10	b	69.54±0.66	b
Vin 5	0.31±0.02	d,e	17.12±0.66	e	35.69±2.10	l	70.73±0.32	c	10.44±0.12	f	29.26±0.08	f	25.24±0.16	c	70.73±0.32	c
Vin 6	0.39±0.02	c	35.02±1.20	b,c,d	33.15±1.10	e	68.95±0.86	b	10.29±0.16	f	31.04±0.21	f	22.86±0.20	b	68.95±0.86	b
Vin 7	0.30±0.02	d,e	28.48±1.18	f	35.05±0.86	j	75.23±1.22	d	8.68±0.55	d	24.76±0.18	d	26.37±0.55	c	75.23±1.22	d,e
Vin 8	0.32±0.01	d	20.55±1.86	e	34.21±0.66	g	70.20±1.44	c	10.19±0.44	f	29.80±0.55	f	24.01±0.86	b,c	70.20±1.44	c
Vin 9	0.20±0.02	b	39.99±1.24	a	32.12±0.48	c	84.61±0.89	g	4.94±0.22	a	15.38±1.66	a	27.18±0.05	c,d	84.61±0.89	h
Vin 10	0.42±0.03	b	28.06±0.88	d	31.22±1.24	b	61.21±0.55	a	12.11±0.65	h	38.79±1.42	h	19.10±0.06	a	61.21±0.55	a
Vin 11	0.31±0.02	b	30.54±1.66	a,b	33.45±0.88	f	73.77±1.18	c,d	8.77±0.88	d	26.22±0.63	e	24.67±0.22	c	73.77±1.18	d
Vin 12	0.45±0.01	f	38.93±0.05	f	33.21±2.20	m	66.59±1.55	b	11.09±0.24	g	33.40±0.48	g	22.11±0.18	b	66.59±1.55	b
Vin 13	0.32±0.01	c	35.14±0.56	c,d	35.40±2.20	i	76.54±0.88	d	8.30±0.10	d	23.45±1.22	d	27.09±0.10	c,d	76.54±0.88	e
Vin 14	0.31±0.02	e	25.78±0.80	b,c,d	34.64±1.20	k	72.81±1.10	c	9.42±0.06	e	27.18±0.92	e,f	25.22±0.22	c	72.81±1.10	c,d

Vintage 2013																
Code	**T. Anth. (gr/lt)**		**AE (%)**		**OD280 (au)**		**MP (%)**		**Dpell (mgr/lt)**		**Dpell (%)**		**Dpep (mgr/lt)**		**Dpep (%)**	
Vin 1	0.35±0.02	d	28.38±1.49	c,d	35.9±0.36	g	71.98±0.07	f	10.05±0.12	f	28.01±0.07	b	25.84±0.23	g	71.98±0.07	f
Vin 2	0.44±0.03	k	31.67±0.22	e	37.3±0.14	h	67.44±0.11	b	12.14±0.01	i	32.55±0.11	f	25.15±0.14	f,g	67.44±0.11	b
Vin 3	0.39±0.02	h	37.00±0.74	f	29.4±0.35	c	65.79±0.14	a	10.05±0.08	f	34.20±0.13	g	19.34±0.27	b	65.79±0.13	a
Vin 4	0.25±0.01	a	27.44±0.51	c	31.1±0.08	e	76.04±0.11	g	7.44±0.05	b	23.95±0.11	a	23.65±0.02	d,e	76.04±0.11	g
Vin 5	0.35±0.01	e	45.53±0.98	h	25.3±0.21	b	69.45±0.25	d,e	7.72±0.13	c	30.54±0.25	c,d	17.57±0.08	a	69.45±0.25	d,e
Vin 6	0.40±0.01	j	38.07±0.54	f	30.6±0.35	d,e	66.88±0.71	a,b	10.13±0.10	f	33.11±0.71	f,g	20.46±0.45	c	66.88±0.71	a,b
Vin 7	0.38±0.02	g	29.38±0.31	d	35.6±0.42	g	69.72±0.27	d,e	10.77±0.04	h	30.27±0.27	c,d	24.82±0.39	e,f,g	69.72±0.26	d,e
Vin 8	0.36±0.02	f	51.97±0.28	i	31.4±0.06	d,e	77.75±0.66	g	6.98±0.04	a	22.24±0.33	a	24.41±1.02	d,e	77.75±0.66	g
Vin 9	0.32±0.02	c	28.49±0.05	c,d	29.5±0.29	c,d	68.38±0.31	c,d	9.32±0.01	d	31.61±0.31	d,e	20.17±0.29	c	68.38±0.30	c,d
Vin 10	0.32±0.01	c	19.98±0.03	a	32.9±0.14	f	68.49±0.02	c,d	10.36±0.04	g	31.50±0.01	d,e	22.53±0.10	d	68.49±0.02	c,d
Vin 11	0.31±0.02	b	24.62±0.68	b	33.7±0.08	f	71.48±0.32	f	9.60±0.09	e	28.51±0.32	b	24.09±0.16	e,f	71.48±0.32	f
Vin 12	0.40±0.01	i	41.96±0.16	g	29.6±0.85	c	68.31±0.89	b,c	9.37±0.02	d	31.68±0.89	e,f	20.22±0.83	b,c	68.31±0.88	b,c
Vin 13	-		-		-		-		-		-		-		-	
Vin 14	0.25±0.02	a	31.93±1.00	e	24.1±0.85	a,b	70.95±1.38	e	6.99±0.07	a	29.04±1.38	c	17.10±0.92	a	70.95±1.36	e

Continued

Vintage 2014																
Code	**T. Anth. (gr/lt)**		**AE (%)**		**OD280 (au)**		**MP (%)**		**Dpell (mgr/lt)**		**Dpell (%)**		**Dpep (mgr/lt)**		**Dpep (%)**	
Vin 1	0.46±0.03	g	40.19±3.57	d,e	36.87±0.68	h	69.85±1.76	d,e	11.11±0.85	c,d	30.14±1.76	d,e	25.75±0.16	d	69.85±1.76	d,e
Vin 2	0.40±0.01	f	24.08±3.10	b	35.70±0.35	g	65.49±4.15	c,d	12.31±1.59	d,e,f	34.50±4.15	e,f	23.38±1.24	c	65.49±4.15	c,d
Vin 3	0.23±0.01	b	28.12±1.27	b.c	27.30±0.21	c	75.10±1.33	f,g	6.79±0.41	b	24.89±1.32	b,c	20.50±0.20	b	75.10±1.32	f,g
Vin 4	-		-		-		-		-		-		-		-	
Vin 5	0.28±0.01	c	43.63±2.87	d,e,f	24.19±0.41	a	73.81±1.69	e,f	6.33±0.29	a,b	26.18±1.70	c,d	17.85±0.71	a	73.81±1.69	e,f
Vin 6	0.34±0.03	e	24.80±2.48	a	35.05±0.67	g,h	61.66±0.79	b,c	13.43±0.03	e,f	38.33±0.80	f,g	21.61±0.71	c	61.66±0.79	b,c
Vin 7	0.59±0.03	i	48.81±0.65	e,f	29.18±0.49	d	58.44±0.72	a,b	12.12±0.01	d,e	41.55±0.73	g,h	17.05±0.50	a	58.44±0.72	a,b
Vin 8	0.22±0.02	a,b	36.39±3.64	c,d	33.13±0.09	f	82.79±1.68	h	5.69±0.57	a,b	17.20±1.68	a	27.43±0.48	e	82.79±1.68	h
Vin 9	0.28±0.02	c	51.61±1.90	f	26.06±0.18	b	79.06±0.25	g,h	5.45±0.03	a,b	20.93±0.25	a,b	20.60±0.21	b	79.06±0.25	g,h
Vin 10	0.53±0.02	h	40.57±2.09	d,e	31.87±0.61	e	60.11±1.61	a,b	12.71±0.26	e,f	39.88±1.61	g,h	19.15±0.87	a	60.11±1.61	a,b
Vin 11	0.31±0.01	d	21.26±4.41	b	36.25±0.18	g,h	72.99±1.51	e,f	9.78±0.49	c	27.00±1.51	c,d	26.46±0.68	d,e	72.99±1.51	e,f
Vin 12	0.21±0.01	a	37.37±6.18	c,d	24.19±0.27	a	78.36±4.15	g	5.23±0.93	a	21.63±4.15	b	18.95±1.21	a	78.36±4.15	g
Vin 13	-		-		-		-		-		-		-		-	
Vin 14	0.60±0.02	i	43.62±0.67	d,e,f	32.09±0.20	e	57.70±0.28	a	13.57±0.18	f	42.29±0.28	h	18.51±0.03	a	57.70±0.28	a

Abbreviations: *T. Anth.:* Total anthocyanins, *AE:* Anthocyanin extractability, *OD280:* Optical density at 280 nm, *%MP*: Contribution of seed tannins to the total phenol content*, Dpell*: Skin tannin concentration, *Dpep:* Seed tannin concentration, *% Dpep / %Dpell*: %contribution to total tannins.

Values represent means of triplicate determinations ± standard deviation.

Values with different letters are significantly different ($p<0.05$).

4.2.5 Conclusions.

In all analyses performed, differences were reported only among the values of the individual vineyards and not among the sub regions examined. Vintage variation was evident in all analyses: 2013 was characterized by lower values of phenolic content and 2012 with the highest. These differences were more likely evident, according to literature, due to water supply, (Kennedy et al., 2000; Lorrain et al., 2011; Zarrouk et al., 2012), delayed maturity (Kennedy et al., 2000; Chira et al., 2011) and reduced ambient temperature (Spayd et al., 2002). In contrast, anthocyanin analysis by HPLC showed reduced content in 2012, more likely due to water supply in agreement to literature (Castellarin et al., 2007; Chacon et al., 2009; Bucchetti et al., 2011; Kyraleou et al., 2015). However, these differences were restricted to malvidin and its derivatives and only when anthocyanins were measured by HPLC. In our results, berry volume and weight did not affect the grape anthocyanin and phenolic content unlike the general belief that smaller berries are more concentrated. Finally, the importance of seed tannins and its contribution to the total tannin content was highlighted with 2012, reported as `poor` vintage due to weather conditions, presenting the highest content and contribution. Therefore, the skin and seed content and ratio to the total tannin content could be used in conjunction to other parameters as indicator of grape maturity and therefore quality.

4.3 Wine analyses.

4.3.1 Wine general analysis.

Classical analyses of the produced wines were performed two months after the completion of the alcoholic fermentation (Table 4.8). Alcohol level (%vol.) ranged between 10.7^{o} and 15.0 ^{o} vol., titratable acidity values were between 3.8 and 7.9 expressed as tartaric acid (g/l) and pH between from 3.12 to 4.24. Concerning color parameters, color intensity ($A_{420}+A_{520}+A_{620}$) and color hue (A_{420} / A_{520}) were found between 3.53 and 13.65 (absorbance units) and 0.47 to 0.94 respectively. Phenolic index was found between 32.6 and 76.1 (absorbance units), while total dry extract between 26.06 to 43.9 g/l. Finally wine density values were found between 0.9860 to 0.9947 g/ml and confirmed that the wines were fermented to dryness. These values fell within the range reported by other researchers for French and Cypriot wines (Chira et al., 2012; Galanakis et al., 2015).

Table 4.8: Classical wine analyses for vintage 2012, 2013 and 2014 (continued in next page).

Vintage 2012								
Code	**Alcoholic content**	**T.A.[1]**	**pH**	**Density**	**Color Int.[2]**	**Color hue[3]**	**Phenolic[4]**	**Dry extr.[5]**
Vin 1	14.5	5.5	3.61	0.9916	7.19	0.64	68.3	40.88
Vin 2	15.0	4.0	3.94	0.9877	8.21	0.70	66.5	32.56
Vin 3	13.4	4.0	3.90	0.9900	3.53	0.81	53.3	33.08
Vin 4	13.1	4.4	4.07	0.9924	4.49	0.84	61.3	38.28
Vin 5	13.2	6.7	3.38	0.9904	8.39	0.52	52.6	33.34
Vin 6	12.7	4.2	3.98	0.9928	5.87	0.76	57.9	38.02
Vin 7	14.6	6.0	3.50	0.9926	11.91	0.51	76.1	43.90
Vin 8	12.3	5.2	3.59	0.9918	5.33	0.60	53.5	33.86
Vin 9	12.8	4.0	4.24	0.9938	4.77	0.94	52.8	40.88
Vin 10	13.0	4.3	3.90	0.9932	8.03	0.71	72.5	40.10
Vin 11	13.0	3.8	3.96	0.9917	8.76	0.69	76.1	36.20
Vin 12	12.1	6.2	3.34	0.9917	8.98	0.47	61.0	32.82
Vin 13	-	-	-	-	-	-	-	-
Vin 14	12.0	5.3	3.63	0.9922	7.83	0.63	67.6	33.86

Continued

Vintage 2013								
Code	**Alcoholic content**	**T.A.[1]**	**pH**	**Density**	**Color Int.[2]**	**Color hue[3]**	**Phenolic[4]**	**Dry extr.[5]**
Vin 1	14.4	5.5	3.74	0.9860	9.56	0.70	55.0	26.06
Vin 2	13.9	4.5	3.53	0.9876	8.09	0.67	48.2	28.40
Vin 3	12.3	4.5	3.48	0.9901	7.54	0.67	43.5	29.44
Vin 4	13.7	4.5	3.71	0.9884	5.99	0.76	42.4	29.96
Vin 5	14.7	5.4	3.45	0.9879	8.34	0.67	42.8	32.04
Vin 6	13.7	7.0	3.32	0.9898	11.24	0.60	44.4	33.60
Vin 7	13.2	4.9	3.49	0.9892	8.12	0.66	46.1	30.22
Vin 8	12.0	5.6	3.18	0.9903	6.37	0.57	40.5	28.92
Vin 9	12.8	5.8	3.76	0.9898	5.04	0.82	37.7	30.48
Vin 10	12.0	5.1	3.36	0.9908	7.75	0.64	46.4	30.22
Vin 11	15.0	4.7	3.82	0.9881	8.94	0.79	51.8	33.60
Vin 12	12.8	6.3	3.12	0.9896	7.34	0.56	38.9	29.96
Vin 13	-	-	-	-	-	-	-	-
Vin 14	12.6	6.0	3.55	0.9907	5.20	0.76	39.3	32.04

Vintage 2014								
Code	**Alcoholic content**	**T.A.[1]**	**pH**	**Density**	**Color Int.[2]**	**Color hue[3]**	**Phenolic[4]**	**Dry extr.[5]**
Vin 1	14.9	5.1	3.44	0.9909	10.79	0.75	57.6	40.36
Vin 2	12.9	5.6	3.62	0.9925	9.93	0.68	46.6	37.76
Vin 3	13.3	4.6	3.80	0.9911	6.57	0.66	38.8	35.68
Vin 4	-	-	-	-	-	-	-	-
Vin 5	10.7	7.9	3.28	0.9937	5.83	0.59	32.6	33.08
Vin 6	13.4	5.2	3.95	0.9947	9.08	0.72	42.6	45.20
Vin 7	12.9	5.8	3.88	0.9925	11.58	0.60	46.1	37.76
Vin 8	12.9	5.4	3.63	0.9915	8.19	0.65	39.8	35.16
Vin 9	12.2	5.6	3.45	0.9911	8.86	0.67	33.5	31.78
Vin 10	13.0	6.1	3.60	0.9928	12.83	0.62	56.9	39.06
Vin 11	14.0	5.0	3.89	0.9925	10.39	0.81	52.3	41.56
Vin 12	11.7	7.7	3.38	0.9925	6.42	0.75	38.4	33.60
Vin 13	13.7	6.9	3.52	0.9907	9.90	0.64	44.8	35.94
Vin 14	14.0	7.1	3.53	0.9907	13.65	0.67	57.4	36.98

[1]Titratable acidity expressed as tartaric acid g/l.

[2]Color intensity: sum of absorbance at 420,520,620 nm.

[3]Color hue: ratio of absorbance at 420/520 nm.

[4]Total phenolic compounds (au), absorbance at 280 nm.

[5]Total dry extract (g/l), calculated indirectly from the specific gravity of the alcohol-free wine.

Vintage 2013 was the most balanced with reduced berry weight, higher color intensity and less phenolics compared to 2012 and greater maturity, higher pH and lower TA in comparison with 2014. Taking into account the unfavorable conditions for Botrytis development that prevailed during that year, allowing delayed harvesting, smoother and less acidic wines were produced.

4.3.2 Spectrophotometric analyses of wine phenolic compounds.

In Table 4.9 are presented the results of the spectrophotometric analyses of wine phenolic compounds. The phenolic content in DMAC index ranged from 231.11 (Vin 9, 2013) to 1104.46 mg/l of catechin equivalents (Vin 8, 2012), with our results being similar to literature (Geldenhuys et al., 2012) for 2013 but twofold increased in 2012. Total tannins by acid hydrolysis ranged from 1.34 (Vin 12, 2013) to 3.31 g/l (Vin 14, 2012) in similar range to values reported by Daudt and Fogaca (2013), for Merlot. Total phenolic content measured by Folin-Ciocalteau ranged from 1.43 (Vin 14, 2013) to 2.79 mg/l of gallic acid equivalents (Vin 11, 2012) among the range reported by Villango et al., (2015) for Syrah and Geldenhuys et al., (2012) for Pinotage. Total tannin content measured by Harbertson assay ranged from 69.90 (Vin 14, 2013) to 508.77 mg/l (Vin 11, 2012) in agreement to Mercurio and Smith (2008), for Syrah and Cabernet Sauvignon, Geldenhuys et al., (2012) for Pinotage and Brooks et al. (2008), for Pinot Noir. Radical scavenging activity (%) ranged from 16.14 (Vin 14, 2013) to 63.04 % (Vin 11, 2012) and DPPH radical-scavenging activity from 8.60 (Vin 12, 2012) to 19.80 of mM trolox equivalents (Vin 1, 2013) results similar to the findings of Fernandez-Pachon et al. (2004). Methyl cellulose precipitation tannin assay (MCP) values ranged from 66.85 (Vin 14, 2013) to 672.95 mg/l of catechin equivalents (Vin 7, 2012).

Table 4.9: Spectrophotometric analyses of wine phenolic compounds for vintage 2012 and 2013 (continued in next page).

Vintage 2012														
Code	**Methyl cell[1]**		**DMAC[2]**		**Acid hydr.[3]**		**Folin[4]**		**Haberson[5]**		**DA (%)[6]**		**DPPH[7]**	
Vin 1	598.25±61.04	f,g	881.92±0.37	*d,e*	2.88±0.18	*a,b,c*	2.32±0.11	*d*	281.35±29.56	*e,f*	52.40±1.96	*c*	10.98±0.95	*c*
Vin 2	588.88±10.53	e,f,g	950.06±10.07	*f*	2.82±0.05	*a,b,c*	2.35±0.02	*d,e*	238.61±13.53	*d,e*	56.93±2.50	*d*	11.90±1.71	*d*
Vin 3	459.93±19.32	c,d,e,f	1016.11±21.72	*g*	2.49±0.04	*a,b,c*	2.08±0.02	*b,c*	187.70±4.70	*c,d*	46.43±3.75	*b*	9.78±0.18	*b*
Vin 4	525.60±87.25	d,e,f,g	768.85±0.59	*b*	2.57±0.14	*a,b,c*	2.28±0.02	*d*	231.55±14.32	*d,e*	54.59±1.76	*c,d*	11.43±0.86	*d*
Vin 5	522.48±52.32	d,e,f,g	919.95±10.59	*e,f*	1.95±0.16	*a*	1.98±0.07	*a,b*	139.95±2.23	*b,c*	48.17±1.10	*b,c*	10.13±0.52	*c*
Vin 6	536.91±37.84	d,e,f,g	1077.28±31.03	*h*	2.27±0.37	*a,b,c*	2.20±0.07	*c,d*	87.65±5.25	*b*	46.28±0.10	*b*	975±0.05	*b*
Vin 7	672.95±46.15	g	1079.69±80.36	*h*	3.18±0.26	*b,c*	2.80±0.01	*f*	500.13±37.09	*g*	60.60±0.10	*e*	12.64±0.05	*e*
Vin 8	227.61±78.12	a,b	1104.46±5.92	*h*	2.47±0.16	*a,b,c*	1.85±0.03	*a*	309.12±45.71	*f*	48.45±1.69	*b,c*	10.18±0.82	*c*
Vin 9	319.44±67.78	b,c	863.69±1.56	*d,e*	2.19±0.30	*a,b*	1.90±0.19	*a*	129.27±28.11	*b*	44.14±0.83	*b*	9.31±0.35	*b*
Vin 10	431.90±52.88	c,d,e	802.05±10.96	*b,c*	3.06±0.27	*b,c*	2.69±0.09	*f*	331.42±16.29	*f*	60.11±0.64	*e*	12.54±0.40	*e*
Vin 11	410.10±80.73	c,d	867.890±3.48	*d,e*	2.82±0.31	*a,b,c*	2.79±0.12	*f*	508.77±32.45	*g*	63.04±0.07	*f*	13.13±0.31	*f*
Vin 12	361.30±7.98	b,c	842.33±15.77	*c,d*	2.23±0.14	*a,b,c*	2.10±0.02	*b,c*	215.67±4.20	*d*	40.60±0.61	*a*	8.60±0.03	*a*
Vin 13	84.00±72.18	a	902.35±12.30	*d,e*	2.66±0.32	*a,b,c*	2.20±0.04	*c,d*	20.95±3.15	*a*	47.55±0.20	*b,c*	10.00±0.01	*c*
Vin 14	345.14±48.97	b,c	698.77±37.62	*a*	3.31±0.47	*c*	2.51±0.04	*e*	300.30±6.96	*f*	56.58±3.65	*d*	11.83±1.78	*d*

[1] tannin concentration determined by the methyl cellulose method expressed as mg/l of epicatechin equivalents.

[2] tannin concentration determined by the DMAC index expressed as g/l.

[3] tannin concentration determined by acid hydrolysis method expressed as g/l.

[4] tannin concentration determined by the Folin-Ciocalteau assay expressed as g/l gallic acid equivalents.

[5] tannin concentration determined by the Adams-Harbertson assay expressed as mg/l of epicatechin equivalents.

[6] tannin concentration determined by the DPPH method expressed as mM trolox.

[7] radical scavenging activity (%).

Values with different letters are significantly different ($p<0.05$). Values are the means of triplicate determinations +/- standard deviation.

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Continued

Vintage 2013														
Code	**Methyl cell[1]**		**DMAC[2]**		**Acid hydr.[3]**		**Folin[4]**		**Haberson[5]**		**DA (%)[6]**		**DPPH[7]**	
Vin 1	149.32±1.19	d	468.73±8.89	*g*	2.52±0.19	*f*	2.20±0.01	*i*	236.29±11.03	*g*	36.13±2.22	*f*	19.80±0.26	*f*
Vin 2	118.20±2.96	a,b,c,d	389.86±18.70	*f*	1.81±0.05	*c,d,e*	1.81±0.04	*f,g,h*	121.00±17.21	*d,e,f*	26.77±2.32	*e*	15.24±0.88	*e*
Vin 3	96.44±0.49	a,b,c	301.76±1.95	*b,c*	1.37±0.19	*a,b,c*	1.74±0.09	*d,e,f*	92.51±4.99	*b*	25.99±1.33	*d,e*	14.85±1.20	*d,e*
Vin 4	118.12±1.27	a,b,c,d	345.92±8.22	*d*	1.71±0.02	*b,c,d,e*	1.65±0.15	*c,d*	159.44±0.96	*e,f*	26.09±1.85	*d,e*	14.90±0.24	*d,e*
Vin 5	115.90±0.88	a,b,c,d	287.10±5.48	*b*	1.65±0.03	*a,b,c,d*	1.64±0.05	*c,d*	118.19±21.54	*d,e*	24.82±1.22	*c,d,e*	14.29±0.33	*c,d,e*
Vin 6	128.18±0.53	b,c,d	240.75±4.10	*a*	2.04±0.02	*d,e*	1.66±0.06	*c,d,e*	120.05±6.83	*d,e*	25.89±2.33	*c,d,e*	14.81±0.63	*c,d,e*
Vin 7	140.11±0.98	c,d	371.79±6.44	*e*	2.15±0.01	*e,f*	1.89±0.05	*g,h*	151.54±1.31	*d,e*	28.06±0.96	*e*	15.87±0.74	*e*
Vin 8	158.56±4.52	d	362.62±9.78	*d,e*	1.79±0.12	*b,c,d,e*	1.70±0.02	*c,d,e,f*	156.21±2.36	*e,f*	22.53±1.85	*b,c,d*	13.17±0.01	*b,c,d*
Vin 9	80.34±3.44	a,b	231.11±8.74	*a*	1.26±0.10	*a*	1.43±0.02	*a*	115.82±2.10	*c,d*	18.90±0.66	*a,b*	11.40±0.68	*a,b*
Vin 10	113.32±2.68	a,b,c,d	348.48±13.63	*d*	1.72±0.02	*b,c,d,e*	1.78±0.02	*e,f,g*	154.91±32.4	*e,f*	27.92±2.12	*e*	15.80±1.20	*e*
Vin 11	135.47±2.56	c,d	317.16±1.35	*c*	2.08±0.15	*d,e,f*	1.92±0.01	*h*	176.37±10.96	*f*	26.59±1.80	*e*	15.15±0.88	*e*
Vin 12	78.78±3.70	a,b	351.47±4.67	*d*	1.34±0.10	*a,b*	1.58±0.01	*b,c*	110.05±23.34	*c,d*	22.27±2.02	*b,c*	13.04±0.24	*b,c*
Vin 13	-		-		-		-		-		-		-	
Vin 14	66.85±1.64	a	308.15±0.98	*c*	1.39±0,07	*a,b,c*	1.49±0.03	*a,b*	69.90±9.20	*a*	16.14±2.82	*a*	10.05±0.88	*a*

[1] tannin concentration determined by the methyl cellulose method expressed as mg/l of epicatechin equivalents.

[2] tannin concentration determined by the DMAC index expressed as g/l.

[3] tannin concentration determined by acid hydrolysis method expressed as g/l.

[4] tannin concentration determined by the Folin-Ciocalteau assay expressed as g/l gallic acid equivalents.

[5] tannin concentration determined by the Adams-Harbertson assay expressed as mg/l of epicatechin equivalents.

[6] tannin concentration determined by the DPPH method expressed as mM trolox.

[7] radical scavenging activity (%).

Values with different letters are significantly different ($p<0.05$). Values are the means of triplicate determinations +/- standard deviation.

In contrast to the results of wine phenolic compounds previously presented, the total tannin content measured by the methyl cellulose precipitation tannin assay, ranged in significant lower values than literature (Sarneckis et al., 2006; Mercurio et al., 2007; Mercurio and Smith, 2008) where values between 1070 to 1140, 1450 to 2300 and 1340 to 4000 expressed as mg/l of epicatechin equivalents were reported respectively. More recently Aleixadre et al. (2018), applied the MCP method in 240 wine samples from different international grape varieties and only Grenache presented values close to our results, but still higher

Wine samples from the three years of the experiment were further analyzed applying the modified Sommers assay. Absorbance values were used to calculate a series of color parameters as described in the Materials and Methods and results underwent statistical analysis (Table 4.10). Comparing our results with literature, Agiorgitiko presented lower values regarding all calculated parameters than Syrah while similar respective values were observed when compared with Cabernet Sauvignon (Mercurio et al., 2007). Wines from vintage 2012 presented higher anthocyanin content, color density and total phenolic content and lower ionization degree (%ion) than the wines obtained from the other two years of the experiment. Wines from vintage 2014 presented the highest ionization degree (%ion) and Chemical age 1 and the lowest anthocyanin content, color density and total phenolics. The results of chemical age 2, color density corrected from SO_2 bleaching and color resistant to SO_2 bleaching were similar among the years.

The effect of vintage is profound in most of the spectrophotometric analyses. Values measured from wines of vintage 2012 were significantly higher than those of 2013 and

in many cases this increase was even twofold higher. Vintage 2013 was reported as an excellent year for the region of Nemea, with cool temperatures, lack of rainfall and reduced botrytis infection unlike 2012; characterized by elevated temperature, heavy rainfall during vintage and increased botrytis infection. Adams and Scholz (2007), estimated that 60% of the wine tannin is extracted from the seeds while only 40% originates from the skins. Skin tannins that frequently are described as “soft” or “ripe,” while seed tannins, are associated with more aggressive and less desirable sensory descriptors like “green” or “hard” (Hernandez-Jimenez et al., 2012). Spectrophotometric analyses presented in Table 4.9, provide few information related to the origin of measured phenolics, alone are weak predictors of wine quality and could be valuable only in combination with other measured wine parameters (e.g. Glories method).

Finally, differences among sub-regions of Nemea were not observed. The differences reported were rather among individual vineyards and not among the different vintages. Comparison among the results obtained by the different phenolic assays employed in this study, failed to show an agreement. This was not surprising since the methods employed are based on different mechanisms for polyphenol determination and they make use of different chemical agents. As far as the methods for tannin determination are concerned, while all measure tannins, they do not assess the same amount or type of tannins. That was more evident in vintage 2012 while in vintage 2013 an agreement was observed but only in few samples (e.g. Vin 1, Vin 9).

Table 4.10: Spectral measures for describing red wine color and phenolics based on the methods of Somers and Evans (1977) color assay.

Vintage 2012								
Code	**Chem. age 1**	**Chem. age 2**	**Ion. (%)**	**Anthocyanins**	**Colour Dens.**	**Col Dens. - SO2**	**Tot. Phenols**	**Colour resist. SO2**
Vin 1	0.27±0.01 b,c	0.09±0.01 d,e	14.7±0.31 c	398.9±7.96 f,g	18.3±0.13 h	13.2±0.09 g	55.8±0.8 b,c,d	2.15±0.05 g
Vin 2	0.32±0.01 d,e	0.08±0.02 f	20.2±1.94 f,g	386.,2±5.42 e	23.1±0.61 i	12.9±0.16 f,g	55.4±0.9 f	2.35±0.07 h
Vin 3	0.26±0.02 b,c	0.07±0.01 c	15.1±0.65 c	303.8±7.85 c	6.3±0.19 a	8.1±0.07 a,b,c	43.4±1.5 a	1.25±0.09 a,b
Vin 4	0.31±0.07 d	0.09±0.01 d	18.1±0.49 d,e	314.3±9.07 c	8.2±0.10 c	9.2±0.05 b,c,d	50.9±0.4 a,b,c	1.59±0.04 d
Vin 5	0.27±0.03 b,c	0.09±0.01 d,e	23.8±0.41 h	336.5±8.51 d	9.8±0.31 e	14.0±0.05 g	43.3±0.5 a	1.79±0.04 e
Vin 6	0.37±0.03 f	0.10±0.04 f	19.8±0.12 f	269.5±1.14 b	7.9±0.12 c	7.9±0.22 a,b,c	47.1±0.4 a,b	1.65±0.06 d
Vin 7	0.28±0.08 c	0.08±0.02 c	20.2±0.48 f,g	466.6±4.19 i	11.5±0.17 g	11.9±0.26 e,f,g	64.9±0.8 d,f	2.04±0.06 f,g
Vin 8	0.38±0.09 f	0.11±0.02 g	19.9±0.53 f	237.8±8.33 a	6.8±0.13 b	7.1±0.10 a,b	43.0±0.4 a	1.60±0.02 d
Vin 9	0.41±0.03 g	0.09±0.06 d,e	13.4±0.92 b	253.3±4.34 a,b	5.9±0.06 a	6.0±0.15 a	43.1±0.8 a	1.33±0.10 b,c
Vin 10	0.34±0.02 e	0.09±0.01 e,f	20.5±0.27 f,g	360.7±1.52 e	10.8±0.14 f	10.4±0.28 d,e	60.7±0.6 c,d,f	2.04±0.10 f
Vin 11	0.34±0.09 e	0.09±0.01 d,e	18.5±0.44 e	387.8±7.27 f	10.4±0.04 f	10.9±0.14 d,e,f	64.6±0.8 d,f	2.12±0.06 f,g
Vin 12	0.21±0.01 a	0.05±0.01 a	21.1±0.68 g	421.7±6.98 h	9.4±0.21 d	9.5±0.06 c,d	50.7±1.3 a,b,c	1.21±0.08 a
Vin 13	0.33±0.01 d,e	0.09±0.01 d	17.4±1.27 d	260.2±8.44 b	6.8±0.24 b	6.7±0.31 a	41.5±0.6 a	1.31±0.01 a,b,c
Vin 14	0.24±0.01 b	0.06±0.01 b	8.7±0.49 a	417.7±10.34 g,h	6.0±0.18 a	9.5±0.33 c,d	55.6±1.0 b,c,d	1.39±0.04 c

Vintage 2013								
Code	**Chem. age 1**	**Chem. age 2**	**Ion. (%)**	**Anthocyanins**	**Colour Dens.**	**Col Dens. - SO2**	**Tot. Phenols**	**Colour resist. SO2**
Vin 1	0.42±0.001 i	0.13±0.015 f	26.4±1.66 h	265.7±26.4 c,d	10.4±0.01 h	9.4±0.76 e,f	44.2±0.5 f	2.20±0.11 e
Vin 2	0.27±0.007 a,b	0.07±0.003 a	20.3±0.14 b,c	363.6±14.8 h	9.1±0.20 f	9.4±0.24 e,f	38.9±1.7 e,f	1.49±0.01 c
Vin 3	0.30±0.004 d,e	0.08±0.001 c	22.9±1.51 e,f	297.4±7.45 e,f	8.5±0.23 d,e	8.4±0.09 c	31.3±2.4 a,b,c,d	1.47±0.01 c
Vin 4	0.31±0.001 e	0.08±0.001 a,b,c	19.1±0.39 b	267.5±1.21 c,d	6.8±0.11 b	7.0±0.12 b	33.9±0.4 b,c,d,e	1.23±0.01 b
Vin 5	0.36±0.007 h	0.11±0.001 e	24.7±0.31 g	260.3±6.13 b,c,d	8.8±0.22 d,e,f	8.5±0.09 c,d	34.5±0.8 b,c,d,e	1.75±0.01 d
Vin 6	0.41±0.006 i	0.15±0.004 g	30.4±0.72 i	257.9±1.04 b,c,d	11.3±0.40 i	10.9±0.11 g	58.1±0.7 c,d,e	2.55±0.02 f
Vin 7	0.30±0.001 c,d	0.08±0.002 b,c	20.7±0.21 c,d	319.3±2.03 f	8.5±0.10 d	8.7±0.15 c,d	38.7±0.1 e,f	1.52±0.02 c
Vin 8	0.28±0.004 b	0.08±0.001 a,b	24.2±0.59 f,g	253.0±1.45 b	7.1±0.13 b	7.1±0.01 b	31.9±0.2 a,b,c,d,	1.17±0.01 b
Vin 9	0.34±0.005 g	0.08±0.001 b,c	17.1±0.03 a	242.4±0.48 a,b	6.0±0.03 a	6.2±0.01 a	30.2±1.1 a,b	1.19±0.01 b
Vin 10	0.34±0.002 f,g	0.11±0.001 e	23.7±0.56 e,f,g	277.7±2.58 d,e	8.8±0.13 e,f	9.0±0.16 d,e	38.2±1.0 e	1.78±0.01 d
Vin 11	0.34±0.003 f	0.09±0.001 d	22.3±0.42 d,e	307.7±5.92 f,f	9.6±0.08 g	9.6±0.07 f	36.9±7.4 d,e	1.74±0.01 d
Vin 12	0.27±0.003 a	0.08±0.001 a,b,c	24.2±0.43 f,g	269.8±0.05 c,d	7.5±0.14 c	7.5±0.06 b	27.9±4.8 a	1.19±0.01 b
Vin 13	—	—	—	—	—	—	—	—
Vin 14	0.29±0.001 c	0.07±0.002 a,b	20.0±0.51 b,c	225.8±5.94 a	5.8±0.03 a	5.8±0.05 a	30.4±0.8 a,b,c	0.97±0.01 a

Vintage 2014								
Code	**Chem. age 1**	**Chem. age 2**	**Ion. (%)**	**Anthocyanins**	**Colour Dens.**	**Col Dens. - SO2**	**Tot. Phenols**	**Colour resist. SO2**
Vin 1	0.36±0.03 a	0.13±0.04 a,b	23.5±0.20 b,c	300.2±4.28 f	10.9±0.14 g,h	12.2±1.56 g,h	50.4±0.5 h	2.41±0.06 f
Vin 2	0.36±0.03 a	0.11±0.01 a	26.1±0.02 b,c,d	270.1±10.20 e	9.6±0.10 f	9.3±0.33 e	39.5±0.5 f	1.93±0.11 d
Vin 3	0.45±0.02 c	0.14±0.01 a,b,c	21.5±1.20 a,b	193.6±6.06 d	6.8±0.14 b	6.7±0.67 b,c	32.3±0.4 c,d	1.73±0.01 c
Vin 4	—	—	—	—	—	—	—	—
Vin 5	0.36±0.03 a	0.14±0.02 a,b,c	32.6±2.44 b,c,d	129.5±3.74 c	5.6±0.22 a	5.3±0.64 a	25.7±0.2 a	1.16±0.01 a
Vin 6	0.70±0.01 f	0.46±0.04 h	70.6±3.87 e	35.2±13.76 a	9.3±0.06 e	9.1±0.25 d,e	58.1±0.6 f	3.36±0.02 k
Vin 7	0.51±0.01 d	0.27±0.01 f	45.0±3.75 c,d	133.1±8.55 c	11.1±0.16 h	11.7±0.19 f,g	38.9±0.3 f	3.23±.0.04 j
Vin 8	0.51±0.01 d	0.20±0.01 e	30.1±1.30 b,c,d	140.4±3.37 c	7.8±0.03 c	7.9±0.55 c,d	32.9±0.4 d	2.18±0.03 e
Vin 9	0.55±0.03 e	0.31±0.02 h	46.2±1.07 d	86.0±6.88 a	8.3±0.13 d	8.9±0.54 d,e	26.6±0.3 b	2.74±0.06 h
Vin 10	0.42±0.04 b,c	0.17±0.01 d	30.6±1.73 b,c,d	263.5±8.34 e	12.5±0.18 i	13.3±0.36 h	50.0±0.1 h	3.12±0.01 i
Vin 11	0.44±0.01 c	0.15±0.06 b,c,d	25.0±1.71 b,c,d	255.9±13.65 e	10.7±0.12 g	10.9±0.40 f	45.2±0.4 g	2.62±0.01 g
Vin 12	0.39±0.01 a,b	0.16±0.01 c,d	31.2±3.38 b,c,d	120.0±8.70 c	5.7±0.08 a	6.4±0.34 a,b	31.7±0.1 c	1.37±0.03 b
Vin 13	—	—	—	—	—	—	—	—
Vin 14	0.36±0.01 a	0.13±0.01 a,b	27.9±1.04 b,c,d	313.1±18.43 f	12.6±0.09 i	13.0±0.08 h	49.9±0.1 h	2.73±0.02 h

Abbreviations: *Chem. Age 1* and *2*: Index of chemical age 1 and 2, *Ion (%):* Degree of ionization of anthocyanins after abolishing SO2 effect on wine color, *Anthocyanins:* Total anthocyanins (mg/lt), *Color dens.*: Color density, *Color dens. -SO2:* Color density corrected from SO_2 bleaching, *Tot. phenols*: Total phenolics (au), *Color resist. SO2:* Color resistant to SO_2 bleaching.

Values are the means of triplicate determinations +/- standard deviation.

Values with different letters are significantly different ($p<0.05$).

4.3.3 Determination of wine anthocyanins by High Performance Liquid Chromatography (HPLC).

Anthocyanin analysis was performed in wine samples from the three years of the experiment and is presented in Table 4.11. 3-O-glucosides of delphinidin ranged from 8.80 (Vin 9, 2013) to 39.20 mg/l (Vin 12, 2012), cyanidin from `not detected` to 9.60 mg/l (Vin 6, 2012), petunidin from 11.29 ((Vin 06, 2014) to 58.93 mg/l (Vin, 12, 2012), peonidin from 10.37 (Vin 6, 2014) to 50.21 mg/l (Vin14, 2014), malvidin from 75.92 (Vin 6, 2014) to 641.88 mg/l (Vin 1, 2012), malvidin-3-O-glucose acetate from 10.92 (Vin 6, 2014) to 76.97 mg/l (Vin 3, 2012) and finally malvidin-3-O-glucose coumarate ranged from 10.38 (Vin 9, 2014) to 105.72 mg/l (Vin 1, 2012).Our results are in agreement with those obtained from the anthocyanin analysis of fresh (non-aged) Agiorgitiko wines by Makris et al., (2006); Petropoulos et al., (2011) and Chorti et al., (2016). Petropoulos et al. (2011), reported total anthocyanins values between 899.2 to 1084.2 mg/l, values closer to our findings but higher than the findings of Makris et al. (2006) (372.5 to 971.4 mg/l) and Chorti et al., (2016) (310 to 350 mg/l).

Table 4.11: Anthocyanin concentration (mg/l) of wine by HPLC, according to Kallithraka et al. (2005).

Vintage 2012														
Code	**Dlp**		**Cyan**		**Pt**		**Pn**		**Mlv**		**Mlv Ac**		**Mlv Coum**	
Vin 1	22,93±0,02	*e*	8,59±0,01	*c*	46,55±1,2	*e*	30,28±1,2	*e*	641,88±10,2	*h*	59,35±2,6	*f*	105,72±6,3	*g*
Vin 2	17,14±0,03	*b,c*	8,41±0,01	*b,c*	35,68±2,3	*d*	23,88±1,6	*d*	501,45±8,9	*c*	54,52±3,3	*e*	85,37±3,2	*f*
Vin 3	11,87±0,01	*a*	*nd*		27,04±2,6	*b*	15,68±1,0	*a*	553,98±10,2	*e*	76,97±2,8	*g*	102,50±6,3	*g*
Vin 4	15,09±0,02	*b*	8,04±0,01	*b*	30,47±2,1	*c*	24,09±1,2	*d*	566,31±20,2	*e*	55,62±2,2	*e,f*	70,29±4,5	*d*
Vin 5	38,65±0,02	*f*	9,10±0,02	*e*	47,52±3,2	*e,f*	29,30±0,8	*e*	496,80±8,6	*c*	32,10±1,3	*b*	56,40±2,6	*a,b*
Vin 6	36,64±0,04	*f*	9,60±0,02	*e*	50,84±0,9	*f*	30,43±1,1	*e*	489,27±6,5	*c*	28,90±1,6	*a*	52,96±3,3	*a*
Vin 7	28,06±0,01	*f*	8,97±0,02	*d*	46,11±2,1	*e*	38,86±1,6	*g*	601,66±12,8	*f*	42,93±6,6	*d*	76,37±4,4	*d,e*
Vin 8	16,24±0,02	*b*	7,97±0,01	*a*	27,25±2,5	*b*	19,56±1,2	*b*	402,54±12,4	*a*	43,43±3,5	*d*	58,66±4,0	*b*
Vin 9	11,05±0,02	*a*	8,17±0,01	*b*	23,23±1,8	*a*	16,40±1,0	*a*	436,35±10,6	*b*	37,23±2,2	*c*	54,41±3,2	*a*
Vin 10	11,71±0,03	*a*	7,91±0,01	*a*	30,49±1,9	*c*	21,65±0,9	*b,c*	513,69±8,9	*d*	53,40±2,9	*e*	66,91±2,9	*c*
Vin 11	18,81±0,02	*d*	8,25±0,01	*b*	36,19±4,0	*d*	24,14±0,5	*d*	519,25±18,6	*d*	58,94±2,8	*f*	57,62±4,2	*b*
Vin 12	39,20±0,04	*h*	9,27±0,02	*f*	58,39±2,6	*g*	36,37±1,6	*f,g*	621,53±10,6	*g*	53,61±3,1	*e*	79,36±2,3	*d,e*
Vin 13	-		-		-		-		-		-		-	
Vin 14	24,63±0,04	*e*	8,30±0,01	*b*	44,73±2,9	*e*	22,44±1,3	*d*	763,29±18,6	*i*	58,68±2,0	*f*	75,02±3,3	*d,e*

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Vintage 2013																
Code	**Dlp**		**Cyan**		**Pt**		**Pn**		**Mlv**		**Mlv Ac**		**Mlv Coum**	
Vin 1	10,30±0,06	*c*	7,79±0,01	*a*	18,99±4,0	*a*	18,49±1,2	*e*	294,67±10,2	*a*	24,41±1,2	*a*	32,74±1,5	*a*
Vin 2	13,96±0,	*f*	nd		30,01±2,1	*g*	16,32±1,2	*c*	535,99±20,6	*f*	61,91±2,8	*g*	47,60±2,2	*d*
Vin 3	12,07±0,06	*d*	7,78±0,01	*a*	23,29±2,6	*d*	14,80±1,0	*b*	428,94±10,8	*d*	42,11±2,1	*e*	39,89±1,9	*b*
Vin 4	9,47±0,04	*b*	*nd*		17,45±3,1	*a*	12,33±0,9	*a*	389,02±10,3	*c*	42,81±2,4	*e*	53,43±2,5	*e*
Vin 5	17,41±0,05	*h*	8,62±0,01	*b*	28,71±3,2	*f*	22,63±1,6	*h*	354,61±6,6	*b*	23,70±2,0	*a*	35,20±2,6	*a,b*
Vin 6	12,10±0,05	*d,e*	8,04±0,02	*a,b*	20,52±2,1	*a,b*	14,67±1,0	*b*	278,41±8,8	*a*	25,92±1,2	*b*	33,93±1,2	*a*
Vin 7	11,95±0,01	*d*	7,64±0,03	*a*	24,46±1,2	*e*	19,07±0,6	*f*	479,15±10,6	*e*	43,49±1,6	*e*	54,68±1,8	*e*
Vin 8	12,31±0,01	*e*	7,71±0,02	*a*	21,14±2,6	*b*	12,79±1,6	*a*	335,26±18,0	*b*	38,09±2,0	*d*	35,53±1,9	*a,b*
Vin 9	8,80±0,01	*a*	*nd*		16,13±1,6	*a*	12,12±1,1	*a*	343,55±14,6	*b*	41,57±1,5	*e*	42,62±2,2	*c*
Vin 10	11,78±0,01	*d*	*nd*		21,55±2,2	*b*	14,04±1,2	*b*	414,53±12,2	*d*	45,23±1,6	*f*	40,93±2,0	*b*
Vin 11	10,79±0,05	*c*	7,83±0,01	*a*	22,37±1,6	*c*	17,32±0,9	*c,d*	364,91±10,8	*b,c*	28,53±2,8	*c*	48,99±3,0	*d*
Vin 12	25,31±0,01	*i*	8,32±0,02	*b*	36,62±3,5	*h*	24,76±1,6	*i*	406,65±10,6	*d*	24,06±0,8	*a*	37,80±2,6	*b*
Vin 13	-		-		-		-		-		-		-	
Vin 14	16,28±0,03	*g*	8,31±0,02	*b*	25,86±2,8	*e*	21,16±1,6	*g*	312,29±8,8	*a,b*	22,18±1,6	*a*	32,47±2,1	*a*

Vintage 2014														
Code	**Dlp**		**Cyan**		**Pt**		**Pn**		**Mlv**		**Mlv Ac**		**Mlv Coum**	
Vin 1	18,40±0,03	*f*	8,09±0,01	*a*	34,96±1,8	*d*	31,079±0,9	*g*	450,59±6,9	*g*	38,71±1,6	*j*	1,60	*g*
Vin 2	16,80±0,	*d*	8,52±0,02	*b*	32,28±1,6	*d*	19,46±1,6	*c*	401,08±10,5	*f*	42,85±2,2	*k*	25,55±1,2	*d*
Vin 3	11,44±0,03	*b*	7,73±0,02	*a*	21,95±1,5	*b*	27,52±1,0	*f*	355,01±10,0	*e*	37,54±1,6	*j*	31,23±1,8	*e*
Vin 4	27,16±0,2	*g*	9,30±0,02	*c*	44,87±2,1	*f*	31,90±1,5	*h*	388,42±10,8	*e,f*	26,30±1,4	*g*	25,16±1,0	*d*
Vin 5	15,57±0,05	*d*	8,17±0,01	*a*	21,30±2,6	*b*	17,74±1,43	*c*	205,57±9,6	*c*	14,87±1,1	*c*	14,21±0,9	*b*
Vin 6	9,37±0,01	*a*	8,23±0,02	*a*	11,29±1,8	*a*	10,37±1,5	*a*	75,92±6,6	*a*	10,92±0,8	*a*	10,47±0,6	*a*
Vin 7	14,35±0,02	*c*	8,73±0,03	*b*	24,65±1,6	*c*	25,27±1,2	*e*	166,78±8,8	*b*	17,64±0,6	*d*	12,92±1,0	*a,b*
Vin 8	11,64±0,01	*b*	8,00±0,02	*a*	19,85±2,2	*b*	26,67±2,2	*e,f*	212,43±8,9	*d*	20,19±1,8	*e*	15,81±0,3	*c*
Vin 9	9,92±0,01	*a*	8,02±0,01	*a*	14,84±3,1	*a*	12,81±1,0	*b*	108,79±6,4	*a,b*	13,09±1,0	*b*	10,38±0,6	*a*
Vin 10	17,16±0,03	*e*	8,54±0,01	*b*	38,86±2,1	*e*	29,41±1,5	*g*	376,76±4,1	*e,f*	29,50±1,6	*h*	23,66±1,2	*d*
Vin 11	12,95±0,03	*b,c*	7,94±0,01	*a*	24,13±1,8	*c*	28,01±1,2	*f*	378,01±6,5	*e,f*	35,16±1,2	*i*	33,47±1,0	*f*
Vin 12	13,82±0,03	*c*	*nd*		20,81±1,6	*b*	21,15±2,0	*d*	184,30±8,8	*c*	13,31±1,0	*b*	14,85±0,6	*b*
Vin 13	-		-		-		-		-		-		-	
Vin 14	34,40±0,02	*h*	*nd*		54,74±1,6	*g*	50,21±2,2	*i*	457,15±16,5	*g*	22,04±0,9	*f*	24,59±1,0	*d*

Abbreviations: *Dlp, Cyan, Pt, Pn* and *Mlv* stand for 3-O-glucosides of delphinidin, cyanidin, petunidin, peonidin and malvidin, respectively. *Mlv Ac:* malvidin-3-O-glucose acetate. *Mlv Coum:* malvidin-3-O-glucose coumarate.

Values represent means of triplicate determinations ± standard deviation.

nd: not detected

Values with different letters are significantly different ($p<0.05$).

In studies performed by Bena-Tzourou and Tsoutsouras, (1992); Lanaridis and Bena-Tzourou, (1997); Makris et al., (2003) and Kallithraka et al. (2005), was reported total anthocyanin content of the studied variety to vary from 33 to 606 mg/l. Their analyses were performed in bottled and aged wines and in some cases blended with other varieties, explaining the great range in values reported by the authors. Comparing Agiorgitiko with other Greek and international grape varieties, Arnous et al. (2002) and

Makris et al. (2003), reported that Agiorgitiko is a variety rich in anthocyanins with higher values than most of the varietals they studied similar to the Syrah and Merlot/Cabernet Sauvignon wine blends.

Makris et al. (2006), analyzed young wines produced and stored in identical conditions from Agiorgitiko, Mandilaria, Xinomavro, Merlot, Syrah and Cabernet Sauvignon sourced from different regions of Greece. Agiorgitiko presented higher anthocyanin values (total and individually) to Xinomavro, Mandilaria and Merlot, similar values to Cabernet Sauvignon and only Syrah presented significantly higher anthocyanin content.

Differences were observed among the three years of the experiment. Higher concentration of individual and total anthocyanin content was reported in the samples of vintage 2012 and the lowest was found in the wines of 2014. It is known that temperature can influence the accumulation of anthocyanins in berries (Spayd et al., 2002; Yamane et al., 2006; Petropoulos et al., 2011) and high temperature (maximum 35^{o}C) after veraison result in anthocyanin degradation (Mori et al., 2007). It has been reported that the accumulation of malvidin-3-O-glucoside and its derivatives is less affected by temperature increase (Pereira et al., 2006; Cortell et al., 2007; Tarara et al., 2008) because of their stable chemical structure called acylated form (i.e., malvidin-3-O-glucose acetate and malvidin-3-O-glucose coumarate), although the concentration of the other anthocyanins might decrease (Tarara et al., 2008).

Our results are in contrast with the above observations, since in 2012 ambient temperature from veraison to harvest was elevated in relation to 2013 and 2014 and the

elevated anthocyanin content was evident in all individual anthocyanins including malvidin and its glucosides.

Botrytis cinerea infection is detrimental to anthocyanin content reducing significantly the skin and wine anthocyanin content (Ky et al., 2012). Such conditions prevailed in vintage 2014, suggesting that the extended infection of grapes by *Botrytis cinerea* resulted in decreased wine colorization. However, such conditions did not occur in 2013, when the *Botrytis cinerea* infection was limited.

In the results previous presented (Table 4.5) skin anthocyanins in 2012 were lower than that of 2013 and 2014 in contrast to the results of wine anthocyanin analyses (Table 4.11), showing elevated anthocyanin content in 2012 against 2013 and 2014. As seen in Table 4.7, the anthocyanin extractability (%AE), was similar among vintages suggesting that the elevated anthocyanin content of wines, was modified during vinification. It is well documented that flavonoids (flavonols and flavanols), phenolic acids, and mainly their derivatives such as caftaric acid favor anthocyanin copigmentation and though color stabilization at all stages of winemaking (Trouillas et al., 2016; Garcia-Estevez et al., 2017). As previously discussed wine phenolics were higher in 2012, than in the wines of the other years of experiment. Therefore, it could be suggested that the elevated content of phenolics resulted to increased anthocyanin copigmentation and complexation and thus to more stable wine color in vintage 2012.

4.3.4 Conclusions.

In our research spectrophotometric analyses results were similar to international grape varieties with the exception of the total tannin content measured by the methyl cellulose

precipitation tannin assay, ranged in significant lower values than literature. Anthocyanin content was elevated in agreement with literature (Makris et al., 2006; Petropoulos et al., 2011; Chorti et al., 2016) confirming that Agiorgitiko is a variety rich in anthocyanins. The effect of vintage was profound in most spectrophotometric analyses and anthocyanin content. Values measured from wines of vintage 2012 were significantly higher than those of 2013 and 2014 and in many cases this increase was even twofold higher. In previous chapter of this study we reported that in 2012 the grape anthocyanin content was reduced against the other years of the experiment. This contradiction could not be explained due to environmental conditions or viticultural parameters but only from loss of wine anthocyanins during storage despite the fact that wines were stored before analysis in controlled conditions.

4.4 Proanthocyanidins analyses of grapes and wine.

4.4.1 Introduction.

Grape phenolic compounds are very important constituents of red wine because, in addition to their antioxidant properties, they contribute to color, astringency and bitterness, oxidation reactions, interactions with proteins and ageing behavior of wines. The aim of our study was to assess the structural characteristics of grape and wine proanthocyanidins of Agiorgitiko variety and to evaluate the influence of the vintage year. Proanthocyanidins (PAs) or condensed tannins are important polyphenolic constituents of red grapes contained in skins and seeds. Seed PAs are composed of (+)-catechin (C), (-)-epicatechin (EC) and (-)-epicatechin-3-O-gallate subunits (ECG), while skin PAs are composed of (+)-gallocatechin, (-)-epigallocatechin (EGC) and (-)-epigallocatechin 3-O-gallate (EGCg) (Prieur et al., 1994; Escribano-Bailon et al., 1995; Quijada-Morin et al., 2012; Li et al., 2014). PAs characteristics and variables such as total concentration, mean degree of polymerization (mDP), subunit composition and distribution are highly correlated to astringency perception and bitterness (Chira et al., 2015). Furthermore, PAs are involved in copigmentation processes with the anthocyanins and the formation of new pigments, which contribute to the stability and definition of red wine color (Cheynier et al., 2006; Gonzalez-Manzano et al., 2009).

Particularly for the grape varieties cultivated in Greece and the wines produced, the PA structural properties have not been examined yet. Only one investigation has been carried out concerning the PA composition of Xinomavro, a variety cultivated in northern Greece (Kyraleou et al., 2015). Therefore, it was thought that characterizing the PA and

their subunit composition of Agiorgitiko (*Vitis vinifera)* grapes and wines would be of great importance in selecting the technological applications which enable the production of high quality wines.

4.4.2 Structural characterization of grape seed and skin PAs.

Figures 4.2, 4.3 and 4.4 show the composition of seed, skin and wine proanthocyanidins (respectively) in polymeric and oligomeric fractions, obtained after phloroglucinolysis. The grape seed PA terminal and corresponding extension subunits determined were (+)-catechin (C), (-)-epicatechin (EC) and (-)-epicatechin-3-O-gallate (ECG), while in this study the presence of (-)-epigallocatechin 3-O-gallate (EGCg) in grape seeds was not observed as reported by other authors (Hanlin and Downy, 2009; Quijada-Morin et al., 2012; Li et al., 2014) (Figure 4.2).

Concerning prevalence, EC followed by C, were the predominant subunits in all samples and in all years of the experiment. EC values ranged from 44% to 52% of total quantified PAs, C values ranged from 18% to 39% and ECG from 15% to 28%. This pattern was observed both in seed oligomeric and in seed polymeric fractions during all three years of the experiment in contrast with other studies (Chira et al., 2009; Chira et al., 2011; Lorrain et al., 2011), who reported significant differences in seed PAs of Cabernet Sauvignon and Merlot grapes among vintages in Bordeaux.

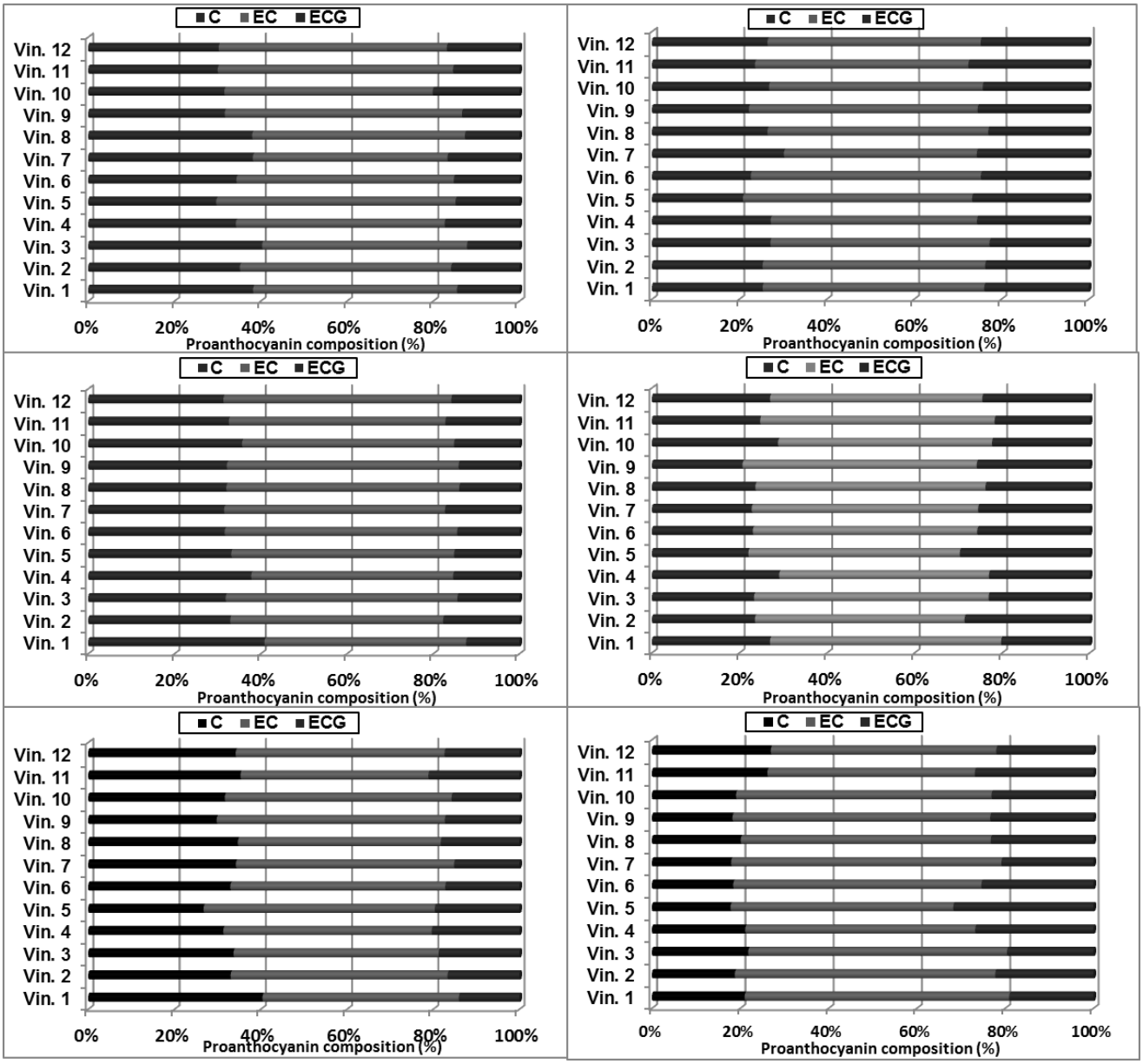

Figure 4.2: Percentage (%) of proanthocyanidin subunits determined in Agiorgitiko grape seeds in oligomeric and polymeric fractions (left and right respectively) for the vintages 2012 (top), 2013 (middle) and 2014 (bottom). Values are the means of triplicate determinations.

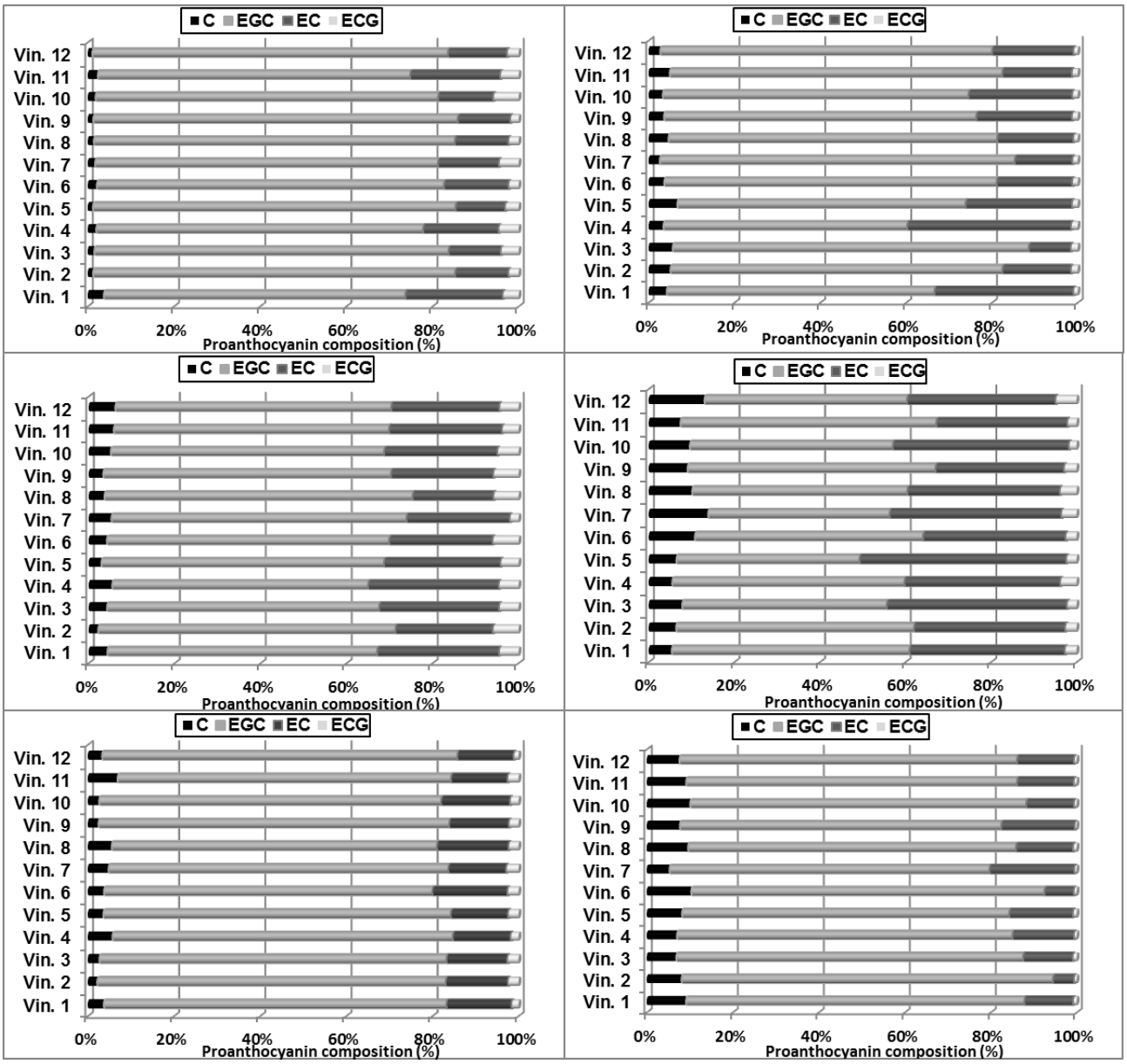

Figure 4.3: Percentage (%) of proanthocyanidin subunits determined in Agiorgitiko grape skins in oligomeric and polymeric fractions (left and right respectively) for the vintages 2012 (top), 2013 (middle) and 2014 (bottom). Values are the means of triplicate determinations.

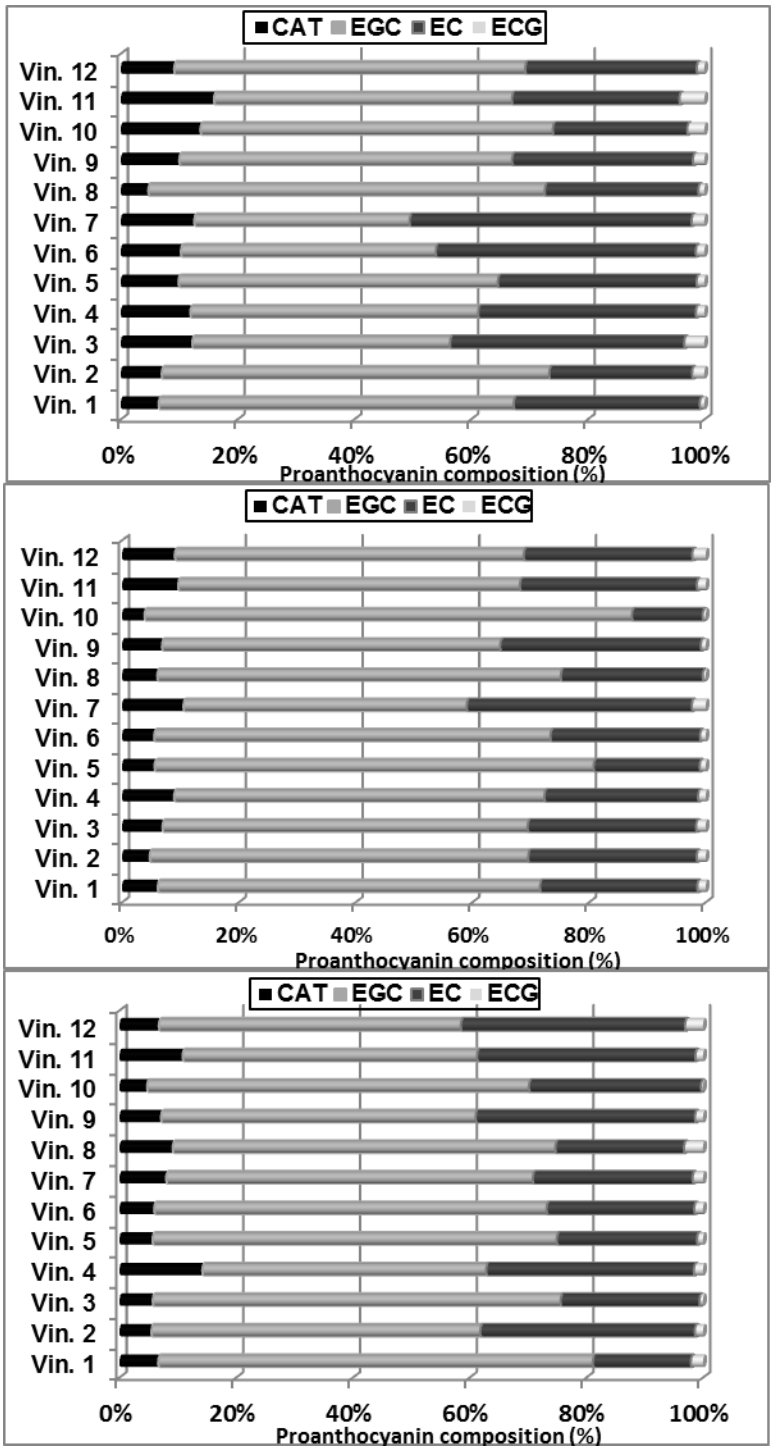

Figure 4.4: Percentage (%) of proanthocyanidin subunits determined in Agiorgitiko wines for the vintages 2012 (top), 2013 (middle) and 2014 (bottom). Values are the means of triplicate determinations

Most previous studies involving different varieties confirm our results that EC is the main subunit in oligomeric and polymeric seed fractions. EC as the predominant subunit has

been reported in seed extracts of Merlot (Chira et al., 2011; Rinaldi et al., 2014), Carmenere, Marzemino, and Syrah (Mattivi et al., 2009) and Xinomavro (Kyraleou et al., 2016) while in Cabernet Sauvignon seed extracts, the results are contradictory (Obreque-Slier et al., 2010; Rinaldi et al., 2014).

The grape skin PAs detected were: (+)-catechin (C), (-)-epicatechin (EC), (-)-epigallocatechin (EGC) and (-)-epi-gallocatechin 3-O-gallate (EGCg). In the skin extracts, EGC was the main subunit, accounting in oligomers for 65% to 85% of total subunits, while in polymers from 42% to 83%, followed by EC (12 % to 31% and 7% to 48% in polymeric and oligomeric fractions respectively) (Figure 4.3). ECG was the least abundant subunit ranging in grape skin oligomers from 3% to 7% and from 1% to 5% in polymers. EGC as a terminal subunit was detected only in few studies (Gagné et al., 2006) or in other studies was entirely absent (Hanlin et al., 2009). Earlier works reported EC as the predominant subunit of skin proanthocyanidins (Monagas et al., 2003; Bordiga et al., 2011) while other authors found C as the main terminal and/or extension subunit in grape skins in a number of varieties (Monagas et al., 2003; Hanlin et al., 2009; Kyraleou et al., 2015).

Lorrain et al. (2011), contacted a similar study investigating the vintage effect on PAs composition of Merlot and Cabernet Sauvignon grapes. During the three years of the experiment (2007-2009), they reported that during vintage 2007, a year characterized by strong water deficiency before flowering, followed by cool temperatures and reduced sun exposure after veraison, a significant increase on the C and EC concentration in grape skin PAs was observed. Reversely, during vintage 2009, characterized by the opposite conditions; the lowest PA accumulation was recorded. In agreement, to their

results, in this study, a significant decrease in PA concentration especially EGC and in less extent C and EC (results not shown) was observed during 2013 unlike the other two years of this experiment (vintage 2012 and 2014). Furthermore, in 2013, the percentage of EC was greatly increased and in some vineyards this increase was higher than threefold (Figure 4.2). This pattern was observed in all samples examined in both fractions (oligomeric and polymeric). As it can be seen in previous session, 2013 was characterized by reduced rainfall from flowering to harvest unlike the other two years of the experiment. Considering the above, vine water deficit before and after veraison could be a possible explanation, in agreement with the findings of Kennedy et al., (2000) and Zarrouk et al. (2012), who in their studies reported accumulation of skin PAs with increased water supply.

Grape maturity had an influence on the grape skin PA subunits, while no significant differences were reported on grape seed PA content. The results showed that the samples with higher sugar content (>13.5^{o} Baume) were characterized by reduced percentage of C, while reduced grape maturity (<12.5^{o} Baume) resulted in the opposite effect (Figure 4.2) in both oligomeric and polymeric fractions. This effect was more evident in 2013 samples. Previous studies of Kennedy et al. (2000) and Chira et al. (2011), reported a gradual decrease in grape seed and skin PAs following veraison until harvest. Although the evolution of the various proanthocyanidin classes and their structural characteristics were not recorder over the ripening period, the findings of this study suggest that maturity plays a crucial role in the evolution of PAs with C being most influenced.

4.4.3 Structural characterization of wine PAs.

Similarly with skin PAs, EGC was the predominant wine subunit ranging from 48% to 76%, followed by EC (from 13% to 48%), C (from 3% to 16%) and ECG (from 1% to 5%) (Figure 4.4). Most previous studies involving wines from different varieties (including Tempranillo, Graciano, Cabernet Sauvignon), reported that C is the main subunit of PAs (Carando et al., 1999; Monagas et al., 2003; Gomez-Alonso et al., 2007; Chira et al., 2011). Unlike these findings, Agiorgitiko PA composition follows a different pattern where EGC is the predominant subunit followed by EC and C.

Quijada-Morin et al., (2012, 2014) reported in their studies a positive correlation between EC concentration and perceived astringency and in agreement with Vidal et al. (2003), a decrease of the perceived astringency / coarseness is reported with elevated EGC concentration. Fernandez et al. (2007), compared Carmenere and Cabernet Sauvignon wines and reported a paradox that even though Carmenere had higher PA content than Cabernet Sauvignon, it was perceived less astringent, possibly due to higher EGC content. Wines produced by Agiorgitiko variety are characterized by smooth, silky tannins and low astringency (Koussisi et al., 2003; Kallithraka et al., 2011), which can be explained by the results of this study since EGC is the predominant subunit of wine PAs.

It is well documented that several winemaking practices influence the extraction of grape PAs into the corresponding wine, (e.g. fermentation temperature, skin maceration, cold maceration, addition of enzymes and oenological tannins) (Bautista-Ortín et al., 2004; Sacchi et al., 2005; Busse-Valverde et al., 2010). In order to have comparable results, the wines used in this study were produced in the same winery and

under similar technological conditions while no exogenous additives apart from dry wine yeasts and potassium sulphate were used.

The results presented in Figures 4.3 and 4.4, indicate a strong relation between the subunit composition of grape skin PAs with that of the corresponding wines while seed PAs seem not to influence significantly wine PA composition. These findings are in agreement with Busse-Valverde et al. (2012), suggesting that skin PAs are more readily extracted than seed proanthocyanidins. Moreover, Adams and Scholz (2007), reported that 96 % of seed PAs remained in seeds after alcoholic fermentation confirming the dominant role of skin PAs in wine. The same authors highlighted the importance of the grape variety, which can greatly affect the proportion of skin or seed-derived PAs in wine. The presented results support the hypothesis that for Agiorgitiko variety, the skin proanthocyanidins have a major contribution to the wine PA content, while the seed PAs are of minor importance.

4.4.4 Mean degree of polymerization (mDP) and percentage of galloylation (% G) of grape proanthocyanidins.

MDP values of skin and seed proanthocyanidins were calculated both in monomeric and polymeric fractions (Table 4.12).The average mDP of the monomeric and polymeric fraction of seeds (1.79 and 7. 43 respectively) was higher than that of skins (1. 17 and 4. 07) This finding is in contrast with the results reported by Bordiga et al. (2011), and Kyraleou et al. (2016), who observed the opposite trend. Oligomeric fractions of skin and seed samples were characterized by significant lower mDP values than the

polymeric fractions in agreement with previous works (Kyraleou et al., 2015) but in contrast with other studies (Prieur et al., 1994; Sun et al., 2013).

The mDP values of seeds during the three years of the study, ranged for oligomeric fractions from 1.63 to 2.02 and for polymeric fractions from 5.84 to 8.57. The corresponding skin values ranged for from 1.35 to 10.69 and from 1.80 to 8.96 for oligomeric and polymeric fractions respectively. In general, mDP values of Agiorgitiko skin extracts were lower than the respective values reported previously for other varieties. Data published by several authors (Chira et al., 2009; Bordiga et al., 2011; Ćurko et al., 2014) reported values of skin mDP between 16.0 and 35.7 for Merlot, 21.9 and 36.6 for Cabernet Sauvignon, 50.2 for Nebbiolo, 30.0 for Plavac mali and 40.0 for Babic (Croatian indigenous varieties). Moreover, seed mDP values in both oligomeric and polymeric fractions were also lower than those reported for Merlot and Cabernet Sauvignon. Due to the high heterogeneity of mDP values reported in literature (Curco et al., 2014; Kyraleou et al., 2016), this parameter could hardly be considered as an index able to characterize and classify the different grape varieties.

Table 4.12: Proanthocyanidin mean degree of polymerization (mDP), of Agiorgitiko grape skins (top) and seeds (midle) (polymeric and oligomeric fractions) and corresponding wines (bottom, next page), for the vintages 2012, 2013 and 2014. Values are the means of triplicate determinations.

Grape skins

Oligomeric fraction	2012	2013	2014
Vin. 1	1.14±0.002 f	1.26±0.004 b,f	1.14±0.005 d
Vin. 2	1.08±0.002 a,b	1.16±0.006 d	1.09±0.001 b
Vin. 3	1.08±0.005 a,b	1.28±0.012 a,b	1.11±0.000 c
Vin. 4	1.19±0.001 g	1.32±0.001 a,c	1.23±0.000 g
Vin. 5	1.09±0.003 b,c	1.18±0.015 d,e	1.13±0.004 d
Vin. 6	1.09±0.002 b,c	1.28±0.007 a,b	1.13±0.001 d
Vin. 7	1.12±0.006 e	1.29±0.011 a,b	1.16±0.002 e
Vin. 8	1.10±0.001 d	1.22±0.006 e,f	1.21±0.001 f
Vin. 9	1.07±0.001 a	1.21±0.053 d,e	1.07±0.121 a
Vin. 10	1.09±0.002 b,c	1.31±0.002 a,b,c	1.09±0.004 b
Vin. 11	1.22±0.006 h	1.33±0.007 a,c	1.25±0.002 g
Vin. 12	1.07±0.003 a	1.35±0.017 c	1.13±0.009 d

Polymeric fraction	2012	2013	2014
Vin. 1	3.82±0.022 b,c,d,e	5.46±0.011 a,b	1.98±0.004 a,b
Vin. 2	3.32±0.052 a,b,c,d,e	6.14±0.061 a,b	2.11±0.119 b,c
Vin. 3	4.44±0.159 d,e	7.72±0.726 c	2.35±0.066 d,e
Vin. 4	2.96±0.002 a,b,c	5.35±0.202 a,b	2.88±0.015 f
Vin. 5	4.20±1.071 c,d,e	7.95±0.698 c,d	2.49±0.070 e
Vin. 6	2.36±0.046 a	6.46±0.226 b	2.41±0.009 d,e
Vin. 7	2.70±0.269 a,b	8.96±0.681 d	2.90±0.085 f
Vin. 8	2.79±0.024 a,b	5.78±0.236 a,b	2.83±0.117 f
Vin. 9	2.88±0.171 a,b,c	5.83±0.137 a,b	2.25±0.043 c,d
Vin. 10	4.68±1.009 e	6.17±0.216 a,b	1.99±0.032 a,b
Vin. 11	3.22±0.497 a,b,c,d	5.20±0.012 a	2.71±0.001 f
Vin. 12	3.38±0.376 a,b,c,d,e,	6.02±0.097 a,b	1.79±0.025 a

Grape seeds

Oligomeric fraction	2012	2013	2014
Vin. 1	1.76±0.005 c	1.66±0.015 d	1.72±0.011 b
Vin. 2	1.96±0.099 h	1.92±0.001 h	1.87±0.003 g,h
Vin. 3	1.80±0.040 e	1.82±0.003 b,c	1.90±0.017 h
Vin. 4	1.63±0.001 a	1.76±0.001 a	1.68±0.003 a
Vin. 5	1.71±0.002 b	1.71±0.014 e	1.81±0.008 e,f
Vin. 6	1.79±0.001 d	1.81±0.014 b,g	1.72±0.012 b
Vin. 7	1.84±0.004 f	1.85±0.014 c	1.72±0.006 b
Vin. 8	1.79±0.014 d	1.75±0.012 a,f	1.77±0.003 c,d
Vin. 9	1.80±0.004 d,e	1.82±0.012 b,c	1.80±0.002 e,f
Vin. 10	2.02±0.001 i	1.78±0.018 a,g	1.87±0.011 g
Vin. 11	1.71±0.003 b	1.72±0.001 e,f	1.76±0.002 c
Vin. 12	1.92±0.003 g	1.66±0.011 d	1.82±0.008 f

Polymeric fraction	2012	2013	2014
Vin. 1	7.61±0.007 b	6.58±0.139 c	5.84±0.057 a
Vin. 2	8.31±0.223 c	7.54±0.159 a,b	6.66±0.073 a,b,c
Vin. 3	7.25±-0.063 a	7.63±0.048 a,b	6.53±0.161 a,b
Vin. 4	8.04±0.042 c	8.48±0.487 d	7.53±0.105 c,d
Vin. 5	7.23±0.071 a	7.03±0.025 a,c	7.80±0.576 d,e
Vin. 6	7.62±0.042 b	7.51±0.029 a,b	6.69±0.095 a,b,c
Vin. 7	8.33±0.095 c	7.56±0.059 a,b	6.84±0.238 b,c,d
Vin. 8	8.15±0.021 c	7.34±0.083 a,b	7.56±0.078 c,d
Vin. 9	7.20±0.111 a	7.13±0.510 a,c	6.62±0.173 a,b,c
Vin. 10	8.16±0.083 c	7.07±0.029 a,c	6.71±0.182 a,b,c
Vin. 11	7.50±0.015 a,b	7.85±0.076 b,d	8.57±0.604 e
Vin. 12	8.16±0.164 c	7.37±0.048 a,b	7.47±0.690 b,c,d

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Wine	2012	2013	2014
Vin. 1	1.80±0.067 b,c	1.63±0.016 c	1.82±0.021 e
Vin. 2	1.77±0.197 b	1.62±0.031 c	2.04±0.054 f,g
Vin. 3	2.48±0.350 d	1.75±0.017 d	1.71±0.021 c,d
Vin. 4	2.09±0.020 e	1.73±0.038 d	2.26±0.027 h
Vin. 5	1.79±0.044 b	1.74±0.031 d	1.40±0.005 a
Vin. 6	2.26±0.041 f	1.56±0.021 b	1.77±0.008 d,e
Vin. 7	2.24±0.002 f	2.27±0.001 g	1.69±0.010 c
Vin. 8	1.38±0.005 a	1.57±0.002 b,c	1.74±0.057 c,d
Vin. 9	2.03±0.015 e	1.79±0.029 d	1.77±0.002 d,e
Vin. 10	2.33±0.023 g	1.43±0.011 a	1.55±0.040 b
Vin. 11	2.41±0.018 h	2.04±0.026 f	2.04±0.013 g
Vin. 12	1.86±0.008 d	1.89±0.060 e	1.97±0.045 f

The effect of the harvest year on mDP was profound in the skin samples only, with higher average values in 2013 compared with 2012 and 2014 (Table 4.12). More specifically, in 2013, mDP values of skin polymeric fractions were almost two folds higher than the corresponding values of 2012 and 2014. An increase was also reported in the mDP values of the oligomeric fraction but less profound, ranging from 2% to 8%. MDP values of seed tannins remained almost unaffected by the harvest year (Table 4.12).

Regarding % G, seeds were characterized by higher average values than skins in both oligomeric and polymeric fractions (10.7 το 3.2 and 6.5 to 0.8 in oligomeric and polymeric fractions respectively) (Table 4.13) in agreement with the findings of other researchers (Rinaldi et al., 2014; Chira et al., 2015; Kyraleou et al., 2016).

The oligomeric fractions of both skins and seeds were characterized by much higher %G than the respective values of polymers, in agreement with Gil et al., (2012). The effect of year was evident only for the skin polymeric fractions, with 2014 samples having lower %G values than the respective 2012 and 2013 samples (Table 4.13).

Finally %P values of skin polymers showed no significant differences among the vineyards and the vintages studied, in contrast with oligomers where a significant decrease of %P was reported for vintage 2013 in all samples studied (Table 4.13). It has been reported by Gil et al., (2012) and Ferre-Gallego et al. (2012), that when weather conditions are propitious for good maturity the amount of prodelphinidins is elevated, indicating grapes of higher quality. However in this study and taking into consideration that vintage 2013, is reported as an excellent year for the region of Nemea, this statement was not confirmed.

4.4.5 Mean degree of polymerization (mDP), percentage of galloylation (% G) and percentage of prodelphinidins (%P) of wine proanthocyanidins.

As it can be seen in Table 4.12, the highest and the lowest wine mDP values (2.48 in Vin 3 and 1.38 in Vin 8 respectively) were obtained during 2012. Moreover, during 2013, the highest mDP value was 2.27 (Vin 7) and the lowest 1.43 (Vin 10) while in 2014, 2.26 (Vin 4) and 1.40 (Vin 5) respectively. Significant variations were observed among the PA structural characteristics of the individual wines of the same harvest year suggesting that even within the same Appellation of Origin Region of Nemea the wines are not characterized by similar chemical composition. The sub regions that can be found within Nemea are probably an important factor that could affect wine sensory properties. In contrast with findings of other researchers (Gil et al., 2012) no relation could be established between grape maturity and wine mDP.

Table 4.13: Percentage of galloylation (%G) of Agiorgitiko grape skins and seeds (top) (polymeric and oligomeric fractions), percentage of prodelphinidins (%P) of Agiorgitiko grape skins (middle) (polymeric and oligomeric fractions) and (P%) and (G%) of the corresponding wines (bottom, next page) for the vintages 2012, 2013 and 2014. Values are the means of triplicate determinations.

Grape skins (%G)

Oligomeric fraction	2012	2013	2014
Vin. 1	3.53±0.285 e,f	3.79±0.013 a,b,c	1.61±0.022 a,b
Vin. 2	2.33±0.131 a,b	5.32±0.370 b	2.51±0.153 d,e
Vin. 3	3.90±0.131 f,g	3.86±0.057 a,b,c	2.55±0.208 d,e
Vin. 4	4.11±0.109 g,h	3.70±0.047 a,b,c	1.72±0.101 a,b,c
Vin. 5	2.98±0.135 c,d	3.88±0.428 a,b,c	2.42±0.151 d,e
Vin. 6	2.38±0.046 b	4.98±0.448 a,b	2.49±0.090 d,e
Vin. 7	4.35±0.320 h	1.89±1.503 d	2.75±0.007 e
Vin. 8	2.23±0.080 a,b	4.99±0.232 a,b	2.21±0.031 b,c,d,e
Vin. 9	1.90±0.021 a	5.07±0.618 a,b	2.09±0.640 b,c,d
Vin. 10	5.60±0.046 i	4.03±0.157 a,b,c	2.01±0.054 b,c,d
Vin. 11	3.59±0.023 f	3.13±0.313 c,d	2.28±0.129 c,d,e
Vin. 12	2.61±0.060 b,c	3.53±0.150 a,c	1.27±0.074 a

Polymeric fraction	2012	2013	2014
Vin. 1	0.71±0.042 a,b,c,d	0.84±0.075 a,b,c	0.68±0.024 b,c,d,e
Vin. 2	0.78±0.008 b,c,d,e	0.73±0.020 a,b	0.60±0.105 a,b,c
Vin. 3	0.66±0.006 a,b,c	0.74± 0.129 a,b	0.76±0.014 e
Vin. 4	1.01±0.001 f	1.03±0.054 c,d	0.54±0.012 a
Vin. 5	0.84±0.028 d,e	0.71±0.007 a,b	0.70±0.011 c,d,e
Vin. 6	0.93±0.017 e,f	0.71±0.052 a,b	0.63±0.040 a,b,c,d
Vin. 7	0.84±0.124 d,e	0.78±0.054 a,b	0.63±0.005 a,b,c,d,e
Vin. 8	0.74±0.005 a,b,c,d	0.99±0.099 c,d	0.71±0.001 c,d,e
Vin. 9	0.79±0.075 c,d,e	0.90±0.015 b,c	0.56±0.012 a,b
Vin. 10	0.70±0.036 a,b,c,d	0.69±0.022 a	0.61±0.071 a,b,c,d
Vin. 11	0.79±0.004 c,d,e	0.78±0.041 a,b	0.64±0.033 a,b,c,d,e
Vin. 12	0.63±0.056 a,b	1.18±0.090 d	0.73±0.001 d,e

Grape seeds (%G)

Oligomeric fraction	2012	2013	2014
Vin. 1	9.67±0.045 c	8.84±0.189 c	9.85±0.077 a
Vin. 2	9.96±0.094 c,d	10.92±0.243 b,d	10.71±0.004 c
Vin. 3	8.34±0.035 a	9.63±0.117 a,c	11.90±0.042 g
Vin. 4	11.88±0.013 i	10.29±0.177 a.b	13.86±0.002 i
Vin. 5	10.19±0.006 d,e	10.51±0.377 a,b,d	12.98±0.034 h
Vin. 6	10.29±0.009 d,e,f	9.65±0.461 a,c	11.78±0.089 f,g
Vin. 7	10.83±0.102 g,h	11.02±0.270 b,d	10.14±0.130 b
Vin. 8	8.69±0.097 a	9.56±0.401 a,c	11.94±0.167 g
Vin. 9	9.11±0.037 b	9.57±0.378 a,c	11.61±0.002 e,f
Vin. 10	11.58±0.027 i	10.35±0.270 a,b	10.23±0.001 b
Vin. 11	10.49±0.138 e,f,g	11.40±0.0426 d	13.76±0.023 i
Vin. 12	10.66±0.192 f,g	10.80±0.567 b,d	10.99±0.006 d

Polymeric fraction	2012	2013	2014
Vin. 1	6.26±0.017 a,b	5.43±0.150 c	6.42±0.003 c
Vin. 2	6.01±0.131 a	6.71±0.201 b	6.41±0.058 c
Vin. 3	6.20±0.011 a,b	5.85±0.113 c,d,e	5.71±0.062 a
Vin. 4	6.46±0.098 b	5.58±0.108 c,d	7.35±0.041 g
Vin. 5	7.05±0.127 c	7.76±0.214 f	8.21±0.131 h
Vin. 6	6.54±0.015 b	6.55±0.060 a,b	7.18±0.089 f,g
Vin. 7	6.55±0.371 b	6.57±0.132 a,b	6.61±0.035 c,d
Vin. 8	6.02±0.029 a	6.24±0.216 a,b,e	6.02±0.132 a,b
Vin. 9	6.99±0.510 c	7.29±0.227 f	7.10±0.166 e,f,g
Vin. 10	6.22±0.213 a,b	6.04±0.152 a,d,e	6.84±0.076 d,e,f
Vin. 11	7.03±0.021 c	5.41±0.202 c	6.84±0.102 d,e,f
Vin. 12	5.98±0.010 a	6.42±0.312 a,b	6.37±0.143 b,c

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Grape skins (%P)

Oligomeric fraction	2012	2013	2014
Vin. 1	63.68±0.136 a	55.45±0.113 a,b,c,d,e	72.66±1.158 c,d
Vin. 2	81.54±0.100 h,i	64.47±0.290 g	77.33±0.247 e,f
Vin. 3	77.60±0.187 f	54.60±0.741 a,b,c,d	75.13±0.149 d,e,f
Vin. 4	72.69±0.265 c	50.74±0.014 a	66.46±0.253 a,b
Vin. 5	81.55±0.489 h,i	58.62±2.351 d,e,f	73.31±0.213 b,c
Vin. 6	76.77±0.037 e,f	56.93±0.285 c,d,e,f	69.52±0.069 b,c
Vin. 7	76.29±0.848 e	57.30±1.443 c,d,e,f	69.80±0.864 b,c
Vin. 8	80.83±0.227 h	61.83±2.035 f,g	64.84±0.311 a
Vin. 9	82.13±0.023 i	60.17±4.083 e,f,g	76.88±1.553 e,f
Vin. 10	75.28±0.093 d	53.72±0.486 a,b,c,d	74.29±1.445 d,e,f
Vin. 11	70.57±0.265 b	52.91±0.765 a,b,c	64.67±0.142 a
Vin. 12	79.65±0.001 g	52.07±0.678 a,b	74.45±4.039 d,e,f

Polymeric fraction	2012	2013	2014
Vin. 1	52.09±0.595 a,b,c,d,e	50.18±0.419 c	61.46±0.246 d,e
Vin. 2	58.59±1.125 e	58.43±0.288 f	70.16±2.333 f
Vin. 3	51.09±0.364 a,b,c,d,e	47.92±1,170 a	58.12±0.842 b,c,d
Vin. 4	44.76±0.033 a	46.67±0.514 a,b	46.48±0.794 a
Vin. 5	46.72±3.338 a,b	51.80±1.133 c	55.33±0.710 b
Vin. 6	54.57±0.166 c,d,e	47.88±0.288 a	56.39±0.409 b,c
Vin. 7	50.19±1.923 a,b,c,d	46.50±0.189 a,b	45.65±1.894 a
Vin. 8	47.09±1.478 a,b,c	41.25±0.444 d,e	49.92±0.679 a
Vin. 9	50.60±3.867 a,b,c,d	51.84±0.025 c	60.14±0.572 c,d,e
Vin. 10	51.00±4.480 a,b,c,d	44.94±1.109 b	63.52±1.275 e
Vin. 11	47.54±4.713 a,b,c	42.07±0.451 e	46.06±0.780 a
Vin. 12	53.96±1.648 b,c,d,e	39.81±0.121 d	56.28±1.149 b,c

Wine (%G)

	2012	2013	2014
Vin. 1	0.72±0.002 a	1.13±0.002 d,e,f	1.30±0.001 e
Vin. 2	1.41±0.002 c,	1.20±0.002 e,f	0.99±0.001 c,d,e
Vin. 3	1.73±0.003 d	1.24±0.002 c,d,e	0.60±0.002 a,b
Vin. 4	0.99±0.001 a,b	0.98±0.001 c,d,e	0.95±0.001 c,d
Vin. 5	1.03±0.001 a,b	0.82±0.001 a,b,c,d	0.90±0.001 b,c
Vin. 6	0.93±0.001 a,b	0.84±0.001 b,c,d	1.04±0.002 c,d,e
Vin. 7	1.26±0.001 b,c	1.39±0.001 f	1.30±0.002 d,e
Vin. 8	0.90±0.001 a,b	0.59±0.001 a,b	2.06±0.001 f
Vin. 9	1.17±0.001 b,c	0.76±0.001 a,b,c	1.03±0.002 c,d,e
Vin. 10	1.46±0.002 c	0.50±0.001 a	0.42±0.001 a
Vin. 11	1.97±0.001 d	1.04±0.001 c,d,e	0.90±0.001 b,c
Vin. 12	0.96±0.002 a,b	1.44±0.001 f	1.82±0.001 f

Wine (%P)

	2012	2013	2014
Vin. 1	48.61±1.649 f	51.81±0.586 e	58.02±0.801 e,f
Vin. 2	52.35±0.116 g	57.35±0.507 g	53.49±1.015 d
Vin. 3	36.21±0.944 b	51.05±0.499 g	55.39±0.609 d,e
Vin. 4	39.90±0.390 c	50.20±0.025 d	37.46±2.232 a
Vin. 5	41.70±2.770 c,d	59.89±0.456 h	60.50±1.751 f
Vin. 6	34.70±3.071 b	56.24±0.467 f,g	54.20±1.086 d,e
Vin. 7	29.23±0.590 a	41.93±0134 a	48.54±4.824 b,c
Vin. 8	55.27±0.814 g	54.88±0.329 f	52.23±2.293 c,d
Vin. 9	44.64±2.754 d,e	49.65±0.197 d,e	47.40±0.422 b
Vin. 10	45.61±0.380 e,f	68.64±0.111 i	55.52±0.469 d,e
Vin. 11	40.08±0,910 c	45.72±1.785 b	36.92±0.186 a
Vin. 12	45.10±0.506 d,e	47.56±1.822 c	37.30±0.315 a

Chira et al. (2011), reported higher mDP values for aged Cabernet Sauvignon and Merlot wines (even threefold), while similar values with the results of this study were observed only for the wines that have been aged for more than seven years. Moreover the studies of Busse-Lalverde et al. (2012) and Quijada-Morin et al. (2012), also reported higher mDP values for Tempanillo (3.1 to 4.3), Monastrell (4.78 to 5.35), Cabernet Sauvignon (5.74 to 7.97) and Syrah (5.00 to 6.74) wines. In addition, Monagas et al. (2003), reported mDP values of 6.9 for Graciano, 9.0 for Cabernet sauvignon and 13.0 for Tempranillo wines while Maury et al. (2003), 5.81 for Merlot and 10.3 for Syrah wines. However, both the above mentioned studies employed thiolysis of wine PAs while in this study the determination took place after reaction with phloroglucinol.

However, the findings of the present study suggest that Agiorgitiko is characterized by significantly lower mDP values compared to most of the international grape varieties irrespectively of the method used for its determination (thiolysis or phloroglucinolysis).

The relation between mDP and wine astringency is well documented but not all studies are in agreement. Chira et al. (2011) and Chira et al. (2012), in their studies on Cabernet Sauvignon and Merlot wines demonstrated a positive relation between wine mDP and perceived astringency. In contrast, in other studies (Quijada-Morin et al., 2012; Wollmann and Hofmann, 2013) mDP values were not correlated significantly with the astringent perception, suggesting that wine astringency might be mainly due to other factors such as PA subunit composition and concentration. Recently, Kyraleou et al., (2016) observed a positive correlation between mDP and astringency only for the shorter tannins suggesting that the size of the molecule is less important for astringency

perception in the case of larger tannins. The presence of galloyl groups (%G), is also a critical factor for astringency. Nevertheless, controversies have been also reported in the literature regarding this issue. %G values correlated positively with perceived astringency in several studies (Chira et al., 2011; Curco et al., 2014) while others either report absence of correlation (Woollmann and Hofmann, 2013; Kyraleou et al., 2016) or negative correlation as in the case of grape seed extracts studied by Chira et al. (2015). In the case of skin EGC, most of the published data are in agreement that it is negatively correlated with astringency perception due to the increase of B ring hydroxylation (Kyraleou et al., 2016; Chira et al., 2015; Quijada-Morin et al., 2012; Vidal et al., 2003).

Concerning wine %G values calculated they ranged from 0.42 % (Vin 10, 2014) to 2.06 % (Vin 8, 2014). Lower %G values were reported during vintage 2012, supporting the hypothesis that due to weather conditions wines were expected to be less astringent (Gil et al., 2012).The highest %P value was reported in Vin 10, 2013 (68.6%) and the lowest in Vin 7, 2012 (29.2). Lower values were reported in 2012, while the opposite trend was observed in 2013. Monagas et al. (2003), reported %G values 2.8 for Graciano and Tempranillo wines while in Cabernet Sauvignon %G was higher (3.4). Maury et al. (2003), also reported much higher %G values for Merlot (8.3) and Syrah (5.1) wines. In contrast, %P values of Graciano, Tempranillo, Cabernet Sauvignon, Syrah and Merlot wines were much lower (8.2, 11.3, 10.6, 19.5 and 12.8 respectively) than those reported in this study (Monagas et al., 2003; Maury et al., 2003).More recently, Chira et al. (2011) and Chira et al. (2012), in their studies regarding the PA composition of aged Cabernet Sauvignon and Merlot wines from 24 different vintages

reported %G values between 0.88 to 6.38 and %P values between 4.31 and 28.0 respectively. Finally, Quijada-Morin et al. (2012), in their studies in Tempanillo wines, reported %P values in the range of 11.5 to 19.9.

In general, %G values were lower while %P values were considerably higher than the respective values reported for international wines measured either after thiolysis (Monagas et al., 2003; Maury et al., 2003) or phloroglucinolysis (Chira et al., 2011; Chira et al., 2012; Quijada-Morin et al., 2012) of wine PAs.

The low Agiorgitiko grape and wine tannin mDP and %G values in combination with the abundance of EGC subunits would be expected to be associated with lower astringency perception. Indeed in a study conducted by Kallithraka et al. (2011), Agiorgitiko appeared to be the less astringent wine among the three native cultivars studied.

4.4.6 Correlations between proanthocyanidin structural parameters and altitude.

Pearson's correlation was employed in an attempt to describe any possible relationship between grape and wine proanthocyanidin structural parameters. It was also of interest to investigate whether the altitude of the vineyard or the average annual rainfall have any influence on grape and wine proanthocyanidin composition (Table 4.14).

Table 4.14: Pearson's correlation coefficients obtained between grape and wine PA structural characteristics[a], altitude and average annual rainfall (n=36).

	%Go	%Gp	%Po	%Pp	%Gw	%Pw	Altitude	Average annual rainfall
Skins								
mDP olig			-0.93**					0.69**
mDP pol		0.56**		-0.47**				0.55**
%G olig								
%G pol				-0.50**				
%P olig				0.51**				-0.80**
%P poly		-0.50**	0.51**					
Seeds								
mDP olig							-0.36*	
mDP pol								
%G olig %G pol %P olig %P poly							0.37*	
Wine								
mDP					0.50**	-0.81**		

**Correlations are significant at the 0.01 level (two-tailed).

*Correlations are significant at the 0.05 level (two-tailed).

[a] mDP olig: mean polymerization degree of proanthocyanidinds in oligomeric fractions; mDP pol: mean polymerization degree of proanthocyanidinds in polymeric fractions; mDp w: mean polymerization degree of proanthocyanidinds in wine; %G olig: percentage of galloyllation of proanthocyanidins in oligomeric fractions; %G pol: percentage of galloyllation of proanthocyanidins in polymeric fractions; %G w: percentage of galloyllation of proanthocyanidins in wine; %P olig: percentage of prodelphinidins of in oligomeric fractions; %P pol: percentage of prodelphinidins of in polymeric fractions; %P w: percentage of prodelphinidins of in wine

As it can be seen from Table 4.14, no significant correlations were obtained between grape and corresponding wine PA structural parameters. Although wine tannins may be of similar molecular size to grape tannins the structural modifications that occur in wines in combination with differences in wine chemical composition make the direct association of grape and wine PA not feasible. In agreement with Cosme et al. (2009), the average calculated mDP of wine PAs is lower than that of the corresponding grape (average value of both fractions) PAs. This could be due to the easier degradation of higher molecular weight proanthocyanidins by cleavage reactions that occur in acidic media like wine (Cosme et al., 2009). These reactions probably dominate in relation to the polymerization reaction of PAs that can also take place simultaneously. Moreover, shorter tannins may be characterized by higher extractability compared to the larger ones which might be retained in the cells (Kyraleou et al., 2016).

In the current study, strong positive correlations where obtained between mDP of skin PAs and %G (in polymeric fraction only) while strong negative values where obtained with %P (in both fractions). It is thus possible that the larger skin PAs are characterized by lower presence of epigallocatechin gallate and higher of epicatechin gallate subunits. Both parameters (%G and %P) are considered critical factors for the ability of tannins to bind proteins.

A similar trend was observed among wine PA structural parameters. Large wine PAs seem to be characterized by higher %G and lower %P.

Regarding altitude, significant correlations where observed only with seed PA structural parameters. Negative values where obtained between altitude and mDP while positive with %G of PAs (in the oligomeric fraction). Regarding average annual rainfall, a

different trend was observed. Only skin PAs were correlated with this parameter while the seed PAs seem not to be affected (Table 4.14). In more detail, positive correlations were obtained between mDp values of skin oligomeric and polymeric fractions while negative between %P (only for oligomeric fraction) and the average annual rainfall indicating a possible higher astringency of grape skins during the more wet years. This finding in is agreement with the results of a previous study where skin extracts from grapes of fully irrigated vines were perceived significantly more astringent than those from non-irrigated ones, suggesting that water supply (irrigation and/or rainfall) is particularly important for wine sensory properties (Kyraleou et al., 2016).

This is a preliminary indication that the altitude may have a significant effect on grape seed proanthocyanidin size and structural characteristics while average annual rainfall on skin PAs. However, more studies are required in order to obtain results that could lead to safe conclusions.

4.4.7 Conclusions.

In conclusion, seed proanthocyanidins of Agiorgitiko grapes were larger, with a higher degree of galloylation and with subunit composition consisting mainly of EC and C. In the skins, proanthocyanidins were shorter and less galloylated, consisting mostly of EGC subunits. The low mDP and %G values, in combination with the abundance of EGC subunit, suggest that Agiorgitiko is a low astringent grape variety, compared to the results presented by other authors for several international grape varieties and the corresponding wines. Concerning skin PAs they might have an important role in the final wine PA composition and content, unlike seed PAs which seem to be of minor

importance. However, a possible connection between grape and the corresponding wine PA composition was not observed. Seed mDP values were influenced by altitude unlike skin mDP values which were significantly affected by the harvest year (average rainfall). More specifically, increased water supply and suitable weather condition for grape ripening augmented mDP values while reduced %P. The results presented, is a first attempt to elucidate the Agiorgitiko grape and wine PA composition since this parameters is of high importance for the wine industry for which the optimization of wine sensory properties remains a priority. However, further research is needed to better understand the complex effects of various environmental parameters on both the structural characteristics of grape PAs and the organoleptic properties of the corresponding wines.

4.5 Amino acid and nitrogen analyses of grape juice and wine.

4.5.1 Introduction.

As discussed in the Literature review session, the nitrogen and AA content and composition of grape juice, affects crucially the wine quality. Nitrogen deficiency has been shown to cause slow and sluggish fermentations, which is why nitrogen addition to the must has become an important part of good winemaking practice. Furthermore, in our research while investigating parameters that could be used as markers of wine quality, to our surprise, research focused in nitrogen and AA in Agiorgitiko grapes and wines, was limited. Therefore, we decided to investigate the nitrogen and AA composition of our samples and likely indicate as parameters of wine quality.

Nitrogen is one of the most important chemical compounds found in the grape must. It is necessary for yeast growth (biomass) and for a number of metabolic functions such as regulation of alcoholic fermentation, sulphur metabolism regulation, flavour compound formation and urea production (Henschke and Jiranek, 1993). A limitation of yeast assimilable nitrogen compounds has been associated with decreased rate of fermentation and an increased duration and frequency of stuck fermentations (Dukes and Butzke, 1998). A general deficiency of assimilable nitrogen compounds has also been related to increased production of hydrogen sulphide (Jiranek et al. 1995b; Spiropoulos and Bisson, 2000). Since nitrogen is necessary for yeast growth, during the exponential phase where *Saccharomyces cerevisiae* experiences the most rapid division/growth, nitrogen utilization is increased. For subsequent stages of the alcoholic fermentation nitrogen utilization is

decreased but still remains necessary for an efficient and complete conversion of sugar to alcohol (Dukes and Butzke, 1998).

The presence of some aroma and flavour compounds in the grape juice and the corresponding wine, have been related to nitrogenous compounds. The nitrogen compounds of must have an influence on the production of esters as amino acids and ammonium determine the pool of intracellular nitrogen, which regulates the metabolic pathways of formation of esters (Henschke and Jiranek, 1993). Esters can be formed from the carbon skeletons of amino acids and their formation is positively correlated with increased must nitrogen and amino acid content (Vos et al., 1978; Bell et al., 1979; Ough and Lee, 1981). Higher alcohols are also directly related to nitrogen metabolism through the Ehrlich pathway (Nykanen, 1986). Total and catabolic production of higher alcohols increased with increasing concentrations of the corresponding amino acids (Schulthess and Ettlinger, 1978). Amino acids play a key role in controlling the pathways of their own formation and thus influence the anabolic formation of higher alcohols (Giudici et al., 1990). The total nitrogen content plays significant role in the formation of higher alcohols with reduced nitrogen content causing increased yield (Ayrapaa, 1968). Finally, Albers et. al. (1996), reported that the nitrogen source and composition of the medium, influences the glycerol yield which was reduced when amino acid were used as the only nitrogen source.

4.5.2 Grape juice yeast assimilable nitrogen content (YAN).

The results regarding yeast assimilable nitrogen (YAN) (or free amino acid nitrogen - FAN) are shown in Table 4.15. Vin 6 (Vintage 2012) presented the

highest value (325.40 mg/l) and Vin 12 (Vintage 2014) the lowest (42.84 mg/l). The annual average YAN values for vintage 2012, 2013 and 2014 were 188.55, 106.11 and 127.26 mg/l respectively.

The concentration of ammonia nitrogen of all vineyards studied was below 2 mg N/l (results not shown). Studies by Bell and Henschke (2005), demonstrated that the range of ammonia nitrogen represents 2-53% of YAN in grape juice depending on the cultivar. These results indicated that Agiorgitiko grapes grown in Nemea region are generally poor in ammonia nitrogen.

Table 4.15: Yeast assimilable nitrogen content of grape must* (mg/l).

Vineyard	Vintage 2012	Vintage 2013	Vintage 2014
Vin 1	124.40±2.60 b	95.11±1.16 e	102.41±0.41 c
Vin 2	230.11±10.02 d,e	118.96±2.66 f	128.19±0.89 d
Vin 3	174,90±3,62 c	151,4±2.67 i	138,66±2,26 d
Vin 4	285,19±4,62 h	101,54± 3.49 e	*nd*
Vin 5	130.77±1.64 b	66.84±1.05 a	79.97±2.17 b
Vin 6	325.40±10.04 g	77.36±0.52 c	229.11±12.86 f
Vin 7	130.04±2.26 b	142.50±0.87 h	125.33±0.21 d
Vin 8	129.21±2.67 b	133.86±1.28 g	127.37±1.10 d
Vin 9	170.34±3.84 c	97.16±2.03 e	178.29±4.78 e
Vin 10	303.81±6.99 f	142.49±0.61 h	126.73±1.63 d
Vin 11	234.09±7.36 e	96.32±0.75 e	211.53±9.52 f
Vin 12	127.21±0.19 b	84.67±2.02 d	42.84±0.02 f
Vin 13	60,43±4,2 a	*nd*	70,63±1,49 b
Vin 14	213.81±10.25 d	71.21±1.02 b	93.35±0.09 b,c
Mean values	188.55	106.11	127.26

Values are the means of triplicate determinations +/- standard deviation.

Values with different letters are significantly different ($p<0.05$).

The importance of nitrogen for yeast growth and alcoholic fermentation is well documented and it is generally agreed that satisfactory fermentation can occur at concentrations over 140 mg YAN/l (Henschke and Jiranek, 1993). In this study, only 12 samples (33%) presented YAN values above the minimum concentration required by yeast as discussed above and only two of the

samples collected at 2013. In annual basis, harvest years 2013 and 2014 contained YAN below the minimum concentration required. YAN was adequate for yeast growth although still close to the minimum requirements only during the harvest year of 2012.

It is clear from the findings of this work that Agiorgitiko grapes grown in Nemea region are characterized by relatively low YAN content and in order to ensure that the alcoholic fermentation will be completed winemakers need to add commercially available nitrogen. To our knowledge, there is no similar study regarding Agiorgitiko (performed in the Nemea region or in any other viticultural region of Greece) and hence due to the lack of relevant results it is not possible to elucidate if this deficiency is related to the cultivar and/or to specific viticultural practices. However, taken into consideration that the grape samples of this study were collected from representative sub-regions which are characterized by different viticultural practices and topography, it seems possible that Agiorgitiko is a cultivar poor in nitrogen

4.5.3 Grape juice amino acid composition.

In this study, eighteen (18) AA were identified and quantified: L-Aspartic acid (asp), L-Glutamic acid (glu), L-Asparagine (asn), L-Serine (ser), L-Glutamine (gln), L-Histidine (his), Glycine (gly), L-Threonine (thr), L-Alanine (ala), L-Arginine (arg), L-Tyrosine (tyr), L-Valine (val), L-Tryptophan (trp), L-Phenylalanine (phe), L-Leucine (leu), L-Lysine (lys), L-Hydroxyproline (hyx), L-Proline (pro) and finally the sum of all amino acids reduced by L-Poline was calculated (total – pro). In Table 4.16, are shown the minimum, maximum and average content of AA (mg/l) measured. The predominant AAs were arginine

representing 27% of the total AA (including proline), followed by proline (21%) and in significant less quantity glutamine (1.8%) and glutamic acid (1.7%). These four AA represented 51% of the total AA content, while all values were among the concentration range reported in the literature (Bell and Henschke, 2005). On the other hand, the AAs with the lowest concentration during all three harvest years were asparagine, glycine and hydroxyproline.

Table 4.16: Free amino acid content (mg/L) of the grape must samples****. Minimum, maximum and average values (left), mean content among the years of the experiment (center) and significance of the effect (right) of vintage and altitude on individual AA content.

	Minimum	Maximum	Average	Vintage 2012	Vintage 2013	Vintage 2014	Vintage	Altitude
asp	9.29	44.95	22.48	24.06±11.3 a	20.39±7.0 a	22.99±9.8 a	ns	ns
glu	35.04	125.19	62.21	77.68±35.3 b	48.46±12.2 a	60.48±19.8 a,b	**	ns
asn	*nd*	1.75	0.27	0.80±2.7 a	0.01±0.01 a	0.01±0.01 a	ns	ns
ser	11.39	66.44	37.71	48.54±13.3 b	30.67±6.3a	33.90±10.8 a	*	*
gln	13.57	291.80	57.92	85.36±72 b	29.57±8.7 a	58.83±28.5 a,b	**	ns
his	9.59	89.35	35.71	54.87±20.7 b	22.61±7.5 a	29.66±10.2 a	**	*
gly	*nd*	12.17	1.76	3.04±5.0 a	1.32±2.3 a	0.92±3.0 a	ns	ns
thr	10.17	102.54	47.52	65.45±22.9 b	34.58±11.7 a	42.55±18.3 a	*	**
ala	19.72	168.63	66.33	92.33±36.6 b	47.99±14.3 a	58.67±24.9 a	**	ns
arg	153.77	2131.69	900.46	1266.62±424.9 b	653.10±276.0 a	781.67±346.1 a	*	*
tyr	*nd*	19.89	7.15	5.49±5.0 a	6.08±1.9 a	9.90±4.0 b	*	ns
val	6.64	53.19	21.55	29.04±10.5 b	16.09±5.8 a	19.53±12.4 a	*	ns
trp	4.42	67.98	23.97	34.73±12.2 b	19.28±20.6 a	17.90±10.6 a	**	ns
phe	8.77	71.95	29.47	38.38±12.7 b	19.91±7.9 a	30.13±16.4 a,b	*	ns
leu	8.26	46.83	23.51	30.49±8.1 b	18.00±6.5 a	22.04±9.1 a	*	ns
lys	*nd*	21.82	7.66	12.78±6.8 b	5.91±4.5 a	4.28±6.7 a	*	*
hyx	0.06	5.37	2.08	2.07±1.8 a	2.51±1.6 a	1.66±1.3 a	ns	*
pro	118.67	1248.08	700.26	763.34±162.3 a	676.47±176.4 a	660.97±270.8 a	ns	**
***total - pro**	298.42	2655.88	1319.09	1800.32±501.7 b	989.79±349.6 a	1167.17±444.1 a	*	*

****Values are the means of triplicate determinations.
*: Significant at 0.05 level.
**: Significant at 0.01 level.
***: Summary of total amino acid content excluding proline
ns: not significant
nd: not detected

The results of this study are in agreement with relevant data of other studies (Huang and Ough, 1991; Spayd and Anderssen-Bagge, 1996; Stines et al., 2000; Bell and Henschke, 2005; Bouzas-Cid et al., 2015) reporting arginine and proline as the most abundant amino acids in grapes. There exists only

one previous work on the amino acid composition of Agiorgitiko grapes (Dourtoglou et al., 1994). Although they measured AA content of the grapes which were stayed for 10 days under carbon dioxide atmosphere, they also reported arginine and proline as the predominant AAs in Agiorgitiko variety. However, aspartic acid, gamma aminobutyric acid (GABA) and alanine were the next most abundant AAs in contrast with the findings of this study, possibly due to the chemical modifications that might take place under storage.

Grape varieties can be classified into categories according to their accumulation of arginine and proline, based on the ratio of these two AAs. This ratio is used to classify grape varieties according to their ability to accumulate either one or the other of these two AAs, so the varieties that have a ratio of proline/arginine <1 are arginine accumulators and vice versa (Garde-Cerdan et al., 2009). Arginine is an important source of nitrogen for the yeast while proline is not utilized during alcoholic fermentation under anaerobic conditions and it can be released during this stage (Martinez-Painilla et al., 2013). However, proline may contribute to berry taste (Torres et al., 2017). Therefore, two cultivars that might have similar total AA concentration; they might differ in their YAN content; the variety that is proline accumulator will be characterized by lower YAN available for the yeast growth (Bell and Henshcke, 2005). Increased levels of arginine, however, could affect wine safety since this amino acid is a precursor of putrescine, a biogenic amine frequently found in wines (Torres et al., 2017). Accumulation of arginine has been reported in Syrah, Petite Verdot, Merlot, Monastrell, Grenache, Chenin Blanc Pinot Noir, Gewurztraminer, Muscat Gordo, Godello,

Treixadura in contrast to Chardonnay, Cabernet Sauvignon, Semillon and Tempanillo varieties (Kliewer, 1970; Kliewer, 1977; Stines et al., 2000; Garde-Cerdan et al., 2009; Lopez et al., 2011; Bouzas-Cid et al., 2015). In this study the proline/arginine ratio in grape must was <1 and more specific for vintage 2012 (0.60) and 2014 (0.85), unlike 2013 when the ratio was 1.03.

The results presented suggest that Agiorgitiko is probably a weak arginine accumulator since the values were below or close to 1, in comparison to the referred studies where the respective ratio values of arginine accumulating varieties were much lower (<0.3). The overall consistency of the results suggests that the profile of amino acid content is mainly determined by genetic factors while environmental parameters and cultivation practices seem to have only a slight modification effect (Stines et al., 2000). Earlier studies by Kliewer (1970), proposed that some cultivars, depending on the maturity, could be either proline or arginine accumulators. In this study, the values of proline/arginine ratio were similar for vintages 2012 and 2014, while during 2013 the ratio was shifted to higher values mainly due to reduced arginine levels. During 2013 greater maturation was achieved due to favored weather conditions, suggesting an influence of maturity on the proline/arginine ratio. However, that influence was not adequate to alter the cultivar from arginine to proline accumulator.

The grapevine rootstock selection has been earlier recognized by Huang and Ough, (1989) and later by Treeby et al. (1998); Holzapfel and Treeby (2007); Lee and Steenwerth (2011) as an important parameter that could affect grape nitrogen content. Vin 1, 7, 9 and 11 were grafted on 41B rootstock while all

other vineyards were grafted on rootstock R110 (Table 3.1). No significant differences were reported among grapevine rootstocks.

The AA composition of the grape must affect the synthesis of volatile compounds and in several studies; the varietal aroma could be partially attributed to the amino acid composition of the grapes (Hernandez-Orte et al., 2009). Wine aroma and flavor is the result of multiple interactions between numerous chemical compounds, derived from the grape, fermentation microflora, secondary microbiological fermentations, ageing and storage of the wine (Styger et al., 2011). Therefore, it is not possible to accurately predict the aromatic profile of the produced wine based solely on its AA composition. However, in many studies the existence of specific aromatic compounds is correlated with their chemical precursors, providing only an estimation of the final wine aroma. The most important odor related compounds (higher alcohols and fatty acids) are produced from valine, phenylalanine, leucine and isoleucine while serine, threonine, methionine, cysteine and aspartic acid could also form odor impacting compounds (Lambrechts and Pretorius, 2000; Marchand et al., 2000; Ardo, 2006; Hazelwood et al., 2008). Regarding the above mentioned AAs, valine, phenylalanine, leucine and aspartic acid were identified and quantified in this study (Table 4.16). The concentration of valine, phenylalanine and leucine was almost 40% higher in 2012 than 2013, the year with the lowest yield. No significant differences were observed regarding the aspartic acid concentration.

The a-nova analysis results presented in Table 4.16, highlighted a strong influence ($p<0.01$) of vintage on glutamic acid, glycine, histidine, alanine and tryptophan and a weaker influence ($p<0.05$) on serine, threonine, arginine,

tyrosine, valine, phenylalanine, leucine, lysine and total AA (without proline). Bouzas-Cid et al. (2018), also reported a significant influence of the harvest year on ten amino acids (glutamic acid, glutamine, histidine, glycine, proline, typosine, methionine, cysteine, tryptophan and γ-aminobutyric acid) which can be attributed to the particular climatic conditions occurring each year. In our study, aspartic acid, asparagine, glycine, hydroxyproline and proline were not significantly influenced by vintage while an influence was reported for all other AA. In addition to the harvest year, altitude significantly influenced the individual AA concentration (Table 4.16). More specifically proline and threonine were strongly influenced ($p<0.01$) while serine, hystidine, arginine, lysine, hydroxyproline and total AA content (excluding proline) were less significantly affected ($p<0.05$).

As seen in Tables 4.15 and 4.16, during vintage 2013 the contents of nitrogen and AAs were decreased compared to the relative contents of the other two vintages. Accumulation of AA is a common phenomenon in plants as a response to stress conditions as water deficit (Less and Galili, 2008). Studies in Spanish grape varieties by Ortega-Heras et al. (2014) and Bouzas-Cid et al. (2015), reported higher AA content during warmer and drier years, with the former, also reporting that irrigation during maturation could increase the AA content. More recently, Torres et al. (2017), compared the impact on AA profiling of deficit irrigation at two time intervals (fruit set to veraison and veraison to maturity) and concluded that warm temperatures and water deficit after veraison enhanced the amount of most AAs. The results reported in this study, suggest that reduced water supply pre-veraison until harvest as occurred during 2013, decreased the AA content of the grapes. In addition, as

seen in Table 3, during vintage 2012, most AA concentrations were elevated in comparison with the respective concentrations of the other two experimental years. Comparing weather conditions, 2012 was the warmest year than the other vintages.

Pearson's correlation was employed in an attempt to describe the relationship between the AA content, average rainfall and temperature during vine growth cycle, calculated from April to September (Table 4.1). As seen in Table 4.17, temperature was positively correlated with nine amino acids (serine, threonine, histidine, alanine, arginine, valine, tryptophane, leucine, lysine and total amino acid content excluding proline) while a negative correlation was observed only with tyrosine. The strongest positive correlation were obtained for serine, histidine, arginine and lysine suggesting that these AA are most probably the most affected by temperature. In addition, it is obvious from the results presented that although there is a general tendency of increasing AA content with temperature, this parameter does not affect the individual AA metabolism with a similar and uniform way. Elevated temperatures have previously been suggested to enhance the biosynthesis of pyruvate (valine, leucine, serine, glycine), oxaloacetate (aspartate, threonine and isoleucine) and 2-oxoglutarate (γ-aminobutyric acid, proline) related compounds (Sweetman et al., 2014). However, some researchers consider that the increase in specific amino acids with temperature might also have resulted from protein degradation in a non-biosynthetic manner (Lehmann et al., 2012). Discrepancies exist in literature regarding the effect of UV exposure on grape amino acid content, owning in part to the different response of the various cultivars (Reshef et al., 2017). For example, filtering the UV-B

irradiance was found to increase the amino acid content of Riesling berries while it did not affect the concentration of Sauvignon Blanc amino acids (Gregan et al., 2012). Reshef et al. (2017), reported an increase of valine, leucine, serine, γ-aminobutyric acid and proline with increased sunlight exposure. However, the direct effect of sun exposure on fruit primary metabolism is not well understood. It is possible that the differences observed are due to the combined temperature effect.

Table 4.17: Pearson's correlation coefficients obtained between grape must amino acid content and average growing temperature and rainfall during vine growth cycle (n=33).

	Temperature		**Rainfall**	
asp	*ns*	.047	*ns*	-.154
glu	*ns*	.268	**	-.458
asn	*ns*	.217	*ns*	-.234
ser	**	.471	*ns*	-.601
gln	*ns*	.223	**	-.461
his	**	.535	**	-.711
gly	*ns*	.242	*ns*	-.221
thr	*	.429	**	-.597
ala	*	.431	**	-.588
arg	**	.462	**	-.608
tyr	*	-.434	*ns*	.132
val	*	.354	**	-.498
trp	*	.422	*	-.424
phe	*ns*	.234	**	-.513
leu	*	.375	**	-.565
lys	**	.505	**	-.458
hyx	*ns*	.109	*ns*	.082
pro	*ns*	.205	*ns*	-.193
total - pro	**	.477	**	-.634

*: Correlations are significant at 0.05 level (two-tailed).

**: Correlations are significant at 0.01 level (two-tailed).

ns: not significant.

In contrast, rainfall was negatively correlated with glutamic acid, glutamine, histidine, threonine, alanine, arginine, valine, phenylalanine, leucine, lysine, hydroxyproline and total amino acid content (excluding proline) while it had a

less significant effect on tryptophane content. Bouzas-Cid et al. (2018), reported that irrigation from June to mid-August reduced the concentrations of cysteine, tryptophan and phenylalanine while it increased proline concentration in Treixadura musts depending on the year. Proline may serve as an osmoprotectant in response to deficit irrigation according to Castellarin et al. (2007), however in the present study it was not affected by rainfall. Phenylalanine was the only amino acid that showed a common tendency in both studies which is the precursor of 2-phenylethanol that provides floral notes to wine aroma (Bouzas-Cid et al., 2018). However, phenylalanine may also be a precursor of Ochratoxin A, a possible carcinogenic compound is humans (Torres et al., 2017). The differences observed between the results of individual amino acids reported by Bouzas-Cid et al. (2018) and this study may be explained by the different climatic conditions and the different cultivar studied (Agiorgitiko). Indeed, previous studies have shown that the metabolism of amino acids in response to water deficit in grapes depends strongly on the cultivar (Deluc et al., 2009). In addition, the grapes in this study were rain-fed and not treated by any irrigation regime since rainfed viticulture is the predominant system worldwide for producing grapes for wine-making purposes. Moreover, in a previous study Bouzas-Cid et al. (2017), reported that irrigation exerted a significant influence on certain amino acids of Albarino grapes depending on the year. In more detail, tyrosine and methionine contents were lower under irrigation than under rain-fed conditions; whereas the opposite was observed for tryptophan. However, the results were not consistent among the different years of the experiment. For example, aspartic acid, proline and tyrosine contents were greater in irrigated

grapes whereas the opposite was observed for methionine during a different year of the experiment. Moreover, they did not observe any significant effect of the irrigation on the amino acids found in musts of the same variety but from a different AOC region. Deluc et al. (2009), observed that a white cultivar subjected to severe water stress did not show differences in the amino acid content, which might indicate that amino acid metabolism in some cultivars, is more resistant to water deficit. In addition, Ortega-Heras et al. (2014), reported that irrigation did not affect the amino acid content of Verdrejo cultivar except for some individual compounds and concluded that maturation stage and mainly climatic conditions are the main parameters that influence the amino acid composition in musts. In several previous studies (Bell, 1994; Stines et al., 2000; Hilbert et al., 2003; Garde-Cerdan et al., 2014) a positive relation between AA content and maturity was also reported. However in the present study grape maturity (expressed as Brix degrees) did not have a significant effect on the AA content (results not shown). This might be due to the maturity stage of the Agiorgitiko grapes, which was similar for all samples since they were collected at harvest.

As it can be seen from the existing literature, the effect of water availability on the concentration of amino acids of grape musts is not clear and consistent among the different cultivars and even for the same cultivar, it varies among the years. The specific climatic conditions during each growing season, the type of the soil and the vineyard management, may be considered as some of the most important parameters affecting amino acid concentration in grapes. In particular, the differences in average water availability and air temperature over the growing season of the vineyards are likely the most important factors

that affect grape maturation and the accumulation of the individual amino acids. Grape vegetative growth, which is depended on climatic conditions as well, might differ from year to year affecting the microclimatic conditions of the cluster zone and reducing the sunlight exposure (Torres et al., 2017). However, in field experiments warmer years are more likely drier years too. In most studies, separation of these two parameters was disregarded. Only Torres et al. (2017), in his experiment, applied during berry ripening two temperature (24^{o}C and 28^{o}C) and three irrigation (full irrigation, early deficit, late deficit) regimes. However, they reported differences only under combination of water deficit and elevated temperature. The present study showed that both average temperature and rainfall were more likely the main factors modulating AA accumulation.

4.5.4 Wine amino acid analysis.

In Table 4.18, are shown the minimum, maximum and average content of wine AA (mg/L) measured. As expected the predominant AA was proline, since it is the predominant AA in grape juice and since it is poorly assimilable by wine yeast under the anaerobic conditions during alcoholic fermentation (Long et al., 2018). The winemaking yeast *Saccharomyces cerevisiae* has been shown to be capable of metabolizing proline when present as the sole nitrogen source (Bandriss and Magasanik, 1979). Even though recent studies by Long et al. (2018), suggested that novel wine yeast able to assimilate proline as nitrogen source is possible, the majority of commercial wine yeast cannot.

Following proline, and in significantly lower concentration, were measured arginine and glutamic acid with all other AAs reported in year average below 10 mg/lt. Our findings come to agreement with Arena et al., (1999); Canas et al. (2008) and Bouzas-Cid et al. (2018), that glutamic acid and arginine were the most abundant amino acids in wines, after proline. Bouloumpasi et al. (2002), analyzed 54 Greek wines and reported alanine, gluatamic acid and arginine as the predominant AAs. Nine of the samples they analyzed were Agiorgitiko and followed the same pattern.

The values obtained in all AAs were in lower concentration than other published data for different grape cultivars (Lehtonen, 1996), Tempanillo (Canas et al., 2008), Alicante Monastrell (Arrieta and Prats-Moya, 2012), Treixadura (Bouzas-Cid et al., 2018) and the study on Agiorgitiko by Bouloumbasi et al. (2002). The variability in amino acids content could be explained on the basis of differences in soil type and composition, fertilization and climatic conditions during growth, degree of maturation and winemaking conditions (Cecchini and Morassut, 2010).

Differences were observed among vintages with 2013 presenting the lowest values in all AAs and 2014 presenting, with the exception of hydroxyproline and proline the highest values. As shown previously in Table 2, on 2013 the grape juice AA content was also lower than the other vintages followed by 2012 and 2014 and though could explain this pattern. In Table 4.19, is presented the percentage of each AA that was consumed during the alcoholic fermentation. Glycine, lysine (vintage 2014), asparagine (vintage 2014) and hydroxyproline presented negative values, suggesting that their content was increased during the winemaking process. Martinez-Rodriguez et al. (2001)

and Bouzas-Cid et al. (2018), also found in wines greater concentrations of individual AAs than in the corresponding grape juice and specifically in glycine, proline and lysine, due to yeast autolysis. Even though are results are in agreement, our vinification process did not include extended ageing on wine lees and therefore the autolysis process did not take place.

Table 4.18: Free amino acid content* (mg/l) of the wine samples. Minimum, maximum and average values (left), mean content among the years of the experiment (right).

	Minimum	**Maximum**	**Average**		**Vintage 2012**	**Vintage 2013**	**Vintage 2014**
asp	*nd*	30.98	4.40		6.68±10.2 b	0.80±0.9 a	5.73± 8.8 a,b
glu	3.34	60.51	18.07		20.49±18.7 b	6.93± 4.1a	26.79±8.9 b
asn	*nd*	8.67	0.60		0.06±0.2 a	*nd*	1.73±2.1 b
ser	0.71	14.57	3.77		4.47±4.2 b	1.92±0.7 a	4.91±1.4 b
gln	*nd*	5.40	1.69		1.54± 1.7 a,b	1.03±0.7 a	2.49±3.7 b
his	1.35	24.93	7.05		5.18±4.5 a	5.34±1.7 a	10.62±2.7 b
gly	*nd*	25.91	5.37		6.58±9.6 a,b	1.48±2.0 a	8.05±3.2 b
thr	0.55	11.48	3.09		3.80±3.4 b	1.45±0.5 a	4.01±5.9 b
ala	*nd*	50.54	7.28		3.80±3.5 a,b	2.56±4.1 a	15.48±21.4 b
arg	*nd*	167.82	24.14		1.,55±20.8 a	6.63±10.8 a	50.23±8.9 b
tyr	*nd*	12.67	2.80		1.86±1.4 a	1.69±0.8 a	4.85±1.3 b
val	0.44	12.20	3.46		3.49±3.5 a,b	2.34±1.1 a	4.56±0.8 b
trp	*nd*	9.80	2.09		2.6±2.9 a	1.53±2.1 a	2.14±2.1 a
phe	0.49	19.11	4.84		5.32±5.0 a,b	2.31±1.0 a	6.90±1.7 b
leu	1.05	17.36	4.37		4.64±4.3 a,b	2.23±0.8 a	6.23±4.5 b
lys	*nd*	34.78	7.61		8,57±1,7 a,b	1.88±1.9 a	12.39±4.2 b
hyx	0.64	6.08	3.28		3.37±1.4 a	3.57±1.3 a	2.91±3.2 a
pro	72.44	984.46	484.76		511.11±156.1 a	469.09±131.1 a	474.08±260.1 a
****total - pro**	14.90	487.30	105.41		109.09±112.0 a,b	43.44±35.8 a	163.70±133.7 b

*Values are the means of triplicate determinations.
**: Summary of total amino acid content excluding proline
nd: not detected
Values are the means of triplicate determinations +/- standard deviation.
Values with different letters are significantly different ($p<0.05$).

With the exception of proline (30.66%), lysine (vintage 2012 and 2013) and tyrosine (63% and 13%) all other AAs were utilized during the winemaking process in great percentage, above 71.68 %. Arginine (97.11%), glutamine (96.82%), threonine (93.52%), asparagine (vintage 2012 and 2013) and tryptophane (90.87%) presented the higher utilization percentages in agreement with Bouzas-Cid et al., (2018). Among vintages differences were observed only on individual AAs and a similar pattern could not be established.

Table 4.19: Individual amino acid utilization (%) during alcoholic fermentation.

	Vintage 2012	Vintage 2013	Vintage 2014		Average
asp	72.24	96.06	75.10		81.13
glu	73.62	85.70	55.71		71.68
asn	92.98	100.00	*neg*		*neg***
ser	90.78	93.72	85.52		90.01
gln	98.20	96.50	95.76		96.82
his	90.56	76.41	64.18		77.05
gly	*neg*	*neg*	*neg*		*neg*
thr	94.20	95.81	90.57		93.52
ala	95.89	94.67	73.61		88.06
arg	98.77	98.98	93.57		97.11
tyr	66.18	72.25	50.97		63.13
val	87.99	85.44	76.67		83.36
trp	92.51	92.08	88.04		90.87
phe	86.13	88.40	77.10		83.87
leu	84.76	87.63	71.72		81.37
lys	32.92	68.15	*neg*		*neg***
hyx	*neg*	*neg*	*neg*		*neg*
pro	33.04	30.66	28.27		30.66
total - pro	93.94	95.61	85.97		91.84

**neg*: negative values.

***neg*: negative values only on 2014.

As previously discussed, the most important odor related compounds (higher alcohols and fatty acids) are produced from valine, phenylalanine, leucine and isoleucine while serine, threonine, methionine, cysteine and aspartic acid could also form odor impacting compounds (Lambrechts and Pretorius, 2000; Marchand et al., 2000; Ardo, 2006; Hazelwood et al., 2008). From the results presented in Table 5, it is shown that in 2013 valine, phenylalanine, leucine, serine, threonine, and aspartic acid presented higher utilization percentage than the other two vintages while in 2014, presented the lowest utilization percentage. Fairbairn et al. (2017), reported a linear correlation between amino acid concentration and the concentration of volatile compounds that are directly derived from these amino acids. Therefore, it could be suggested that in 2013, higher AA utilization could correspond to higher concentration of volatile compounds and the opposite for 2014. Yet, as seen in Table 4, in

2013 was reported the lower AA concentration than the other two vintages and as result even though proportionally the AA utilization was higher, possible higher production of volatile compounds would be netted by the reduced precursor AA content.

4.5.5 Conclusions.

On the basis of the above findings, it is highlighted the importance of grape cultivar on the composition and content of grape AA. Agiorgitiko is a cultivar with reduced YAN and ammonia nitrogen, in most cases marginally adequate for successful alcoholic fermentation. Most vineyard characteristics evaluated in our study (e.g. rootstock, vine density, maturity) had a minor impact and only on individual AAs. Furthermore our results showed that rainfall and temperature during vine growth cycle influenced grape AA composition. For the viticultural region of Nemea, the AA content of Agiorgitiko grapes was negatively affected by rainfall from veraison till harvest while elevated ambient temperature had the opposite effect. Those findings come in contrast with research on other cultivars highlighting its importance. Since, this is the first research focusing solely on Agiorgitiko and only in Nemea wine region, it is not clear if the impact of cultivar prevails over environmental conditions and at which extent. Furthermore, we reported that altitude, parameter that is strongly related to environmental conditions, significantly influenced individual AA content. In field experiments, isolation of individual environmental parameters is not always feasible resulting to contradictory results. It is therefore suggested that the accumulation of individual AAs is crucially affected by the combination of grape cultivar and climatic conditions during growing season. Due to the importance of grape nitrogen and AA composition

and content, further research is required to better understand the implication of these parameters, introducing viticultural methods and practices that could manage their accumulation.

Concerning wine amino acid content, the results of our findings come in agreement with the international literature. No significant differences were reported among Agiorgitiko and other international grape varieties concerning the assimilation of AAs during alcoholic fermentation. All differences reported were related with the initial grape juice AA content suggesting that parameters that affect grape juice AA composition and content are crucial.

4.6 Fuzzy logic multi criteria decision making system for Grape-Wine model establishment.

A fuzzy logic multi criteria decision making (FMCDM) system for ranking approach based on fuzzy set theory was developed in order to develop a simplified approach to estimate the relationship among wine quality, as defined by the tasting panel, and analytical parameters. The system was based on Mamdani Fuzzy interface (Mamdani and Assilian, 1975) with eight inputs and one output. The implementation was performed with the software package Matlab using the Fuzzy Logic Toolkit (Jang et al., 1997). The inputs are the grape quality parameters defined by the oenologist experts participating in the experiment, based on their experience and on literature. The panel of experts consisted of Professors in Oenology and Viticulture from Agricultural University of Athens (AUA) and Technological Educational Institution of Athens (ATEI) but also of oenologist/viticulturist with all three of the following qualifications a) Bachelor in Oenology and/or Viticulture b) Master in Science in Oenology and/or Viticulture and a c) minimum working experience of 10 years in the wine region of Nemea as wine production managers and/or head oenologist/viticulturist. In particular, we used average berry volume (BV), total soluble solids (TSS) expressed as ^{o}Baume, Botrytis infection, Optical Density (OD 520), anthocyanin extractability (EA), seed colorization (SC), pH and skin phenolic content (Dpell). Each input was represented by a class that was defined by a set of threshold values. Thresholds were separated in four (4) categories for all parameters; except TSS where five (5) categories were used. Those values were determined by

viticulture and wine-making experts according to their experience based on literature and further modified to Agiorgitiko grape variety (Table 4.20).

Table 4.20: Threshold values of quality parameters.

Parameters	Unit	Thresholds				
		Very Poor	Poor	Good	Very Good	Excellent
Berry Volume (BV)	mL	> 240	190 - 240	150 - 190		< 150
Total Soluble Solids (TSS)	°Baume	< 11.6	11.6 - 12.8	12.8 - 13.3 or > 14.0	13.3 - 13.6	13.6 - 14.0
Botrytis infection	%	> 5		0.25 - 5		0 - 0.25
Optical Density (OD 520)	-	< 8.0	8.0 - 12.5	12.5 - 16.5		> 16.5
Anthocyanin extractability (EA)	%	> 59.0	39.0 - 59.0	21.0 - 39.0		< 21.0
Seed colorization (SC)	-	Green	Half green / half brown	Faint green		Brown
pH	-	> 3.86 or < 3.20	3.20 - 3.39 or 3.77 - 3.86	3.47 - 3.77		< 3.47 and > 3.39
Skin phenolics (Dpell)	mg/L	< 7.5	7.5 - 10.5	10.5 - 12.0		> 12.0

The different inputs were quantified into numbers ranging from zero (very poor) to one (excellent). A membership function of each input was developed and the membership curve for each input is presented in Figure 4.5.

The fuzzy rules were generated, using the different fuzzy sets of the 8 inputs indicators and their classes (Serge, 2001; Wu et al., 2001). However, the different nature of the inputs was evaluated to determine the influence of each parameter to the final decision. Each input does not take the same priority in the FMCDM system. The knowledge of the experts was utilized in order to evaluate the importance of each parameter. The experts employed a linguistic weighting set according to certain technical parameters.

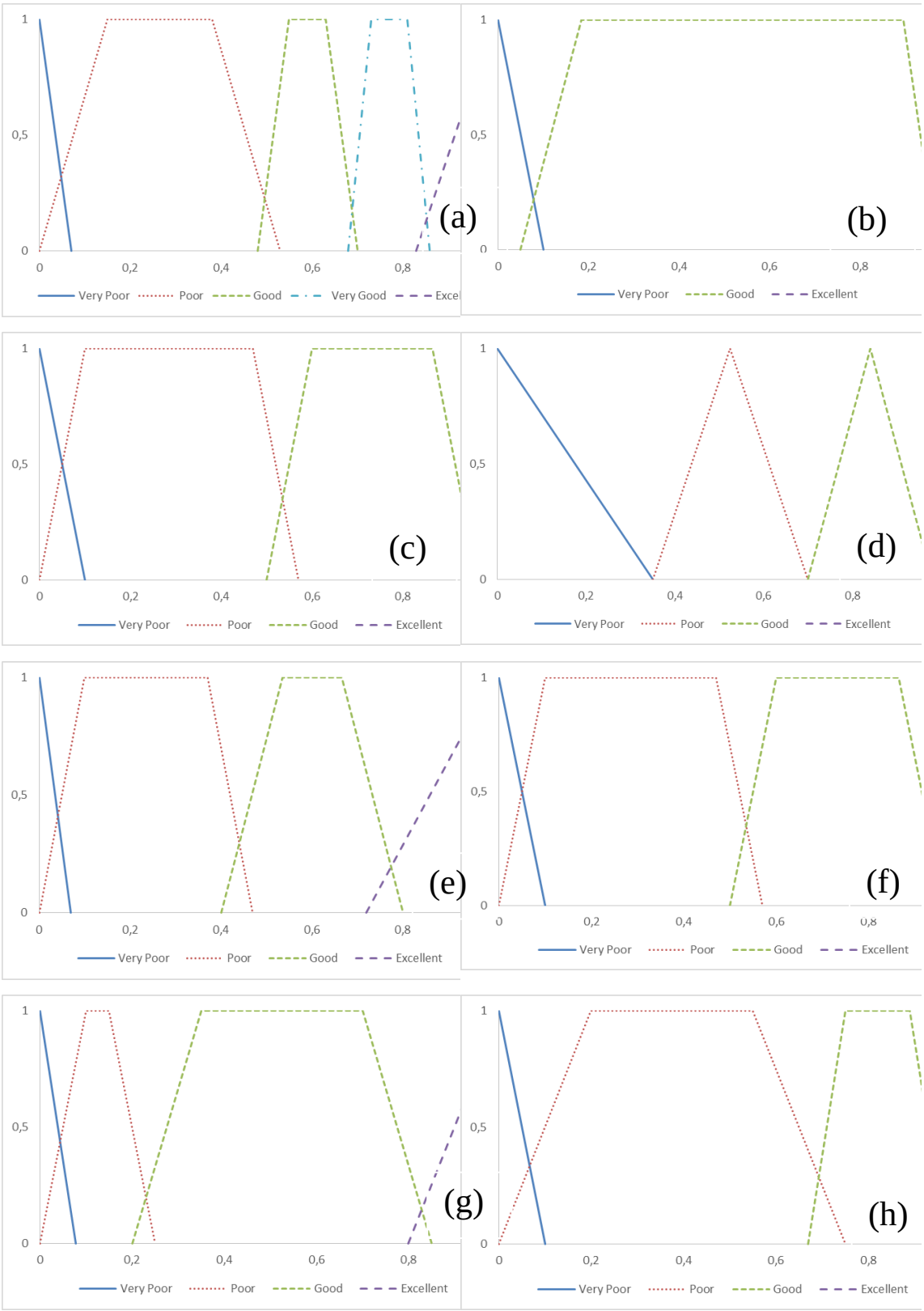

Figure 4.5: Membership curve of each input: (a) Total Soluble Solids (^{o}Baume), (b) Botrytis infection (%), (c) Optical Density (OD520), (d) Anthocyanin extractability (%), (e) Berry Volume (mL), (f) Seed colorization (SC), (g) pH and (h) skin phenolics (Dpell) (mg/l) Range from `zero` (very poor) to `one` (excellent).

More particularly, BV received high weighting because as a general rule, smaller berries are positively related to wine quality, having more concentrated solutes.

Furthermore, the increased skin/pulp ratio in smaller berries has a positive effect on wine sensory characteristics (Hardie et al., 1997). BV is mainly influenced by environmental conditions and viticulture parameters and is an important parameter for assessing vineyard and grape quality. Another parameter that received high weighting was pH that affects almost all chemical reactions taking place during vinification and ageing of the wine, but also has a direct effect on wine organoleptic properties. Elevated pH level leads to lighter colored wines, dull, without nerve and reduced ageing potential. In contrast, low pH wines are acidic, unpleasant and correlated with unripe aromas and flavor (Ribereau-Gayon et al., 2000). Finally, TSS was also considered as important parameter from the experts as it is well documented that maturity has a marked effect on wine quality and TSS are often used by winemakers as indicator of grape quality. Lower TSS are related to unripe grapes with higher acidity, lower aroma and phenolic composition (Fang and Qian, 2006; Pérez-Magariño and Gonzalez-San Jose, 2006). Excessive sugar ripeness results to wines often descripted as `dead fruit wines` referring to high alcohol, unbalanced wines with `cooked`, `jammy` aromas.

Important parameter, but a little less than the above mentioned (BV, pH, TSS) is SC. Changes in seed development are highly reflected in the sensory attributes of grapes (Fredes et al., 2010). The external appearance of the seed coats may be used as an additional indicator of the overall berry

ripeness and though the wine quality (Ristic and Illand, 2005). Darker seed color is appreciated by winemakers as a marker of lower seed tannin extractability and improvement in astringency (Fredes et al., 2010). Dpell is also significant and follows in weighting. The importance of tannins in the sensory properties of red wine is well documented, particularly with respect to astringency and bitterness. Astringency is a tactile sensation described as drying, roughing or puckering mouth feel that results from the interaction of tannins with salivary proteins (Mc Rae et al., 2010). Recently Petropoulos et al. (2017), highlighted the relation between skin and wines tannins in Agiorgitiko, supporting the importance of skin phenolics in wine quality.

Botrytis infection, EA and OD520 received the lowest weighting from the experts, because even if they are important parameter for wine quality, they do not play the most significant role. Botrytis infection development deteriorates grape quality and the organoleptic properties of the produced wines. Anthocyanins are a family of phenolic compounds directly related to red wine colour. They are located in the grape skins of red grape varieties and are extracted into wine during vinification. Even though the composition and content of grape anthocyanins is important, highly-colored grapes do not always produce highly-colored wines since the EA differs among vineyards and grape varieties (Romero-Cascales et al., 2005). Finally, wine color is influenced by many parameters during vinification (e.g. SO_2 additions, extraction time, temperature). Furthermore, color modification during vinification and ageing, is a complicated chemical process influenced by parameters that are difficult (or impossible) to predict. Therefore, as a grape

parameter it is surely important but still not very critical for the prediction of wine quality. The final priority vector is presented in Table 4.21.

The intensity of each parameter was rated as Very Low (numeric value equal to 1), Low (numeric value equal to 2), Medium (numeric value equal to 3), High (numeric value equal to 4) and Very High (numeric value equal to 5). The answers of all experts were summed and a matrix (A) was prepared. The matrix A was normalized by dividing each value by the sum of the matrix's column. The priority vector was calculated by Equation 1.

$$w(A - nI) = 0 \quad (1)$$

Where w is the priority vector matrix and the sum of all vectors is equal to one, `I` is the identity matrix and `n` is the number of parameters.

Table 4.21: Linguistic weighting set of grape parameters used in the FMCDM system.

Parameters	**Decision - makers**				**Priority vector**
	Expert 1	Expert 2	Expert 3	Expert 4	
Berry Volume (BV)	High	Very High	Very High	Very High	0.20
Total Soluble Solids (TSS)	High	Very High	Very High	Very High	0.20
Botrytis infection	Very Low	Very Low	Low	Very Low	0.05
Optical Density (OD 520)	Very Low	Low	Very Low	Very Low	0.05
Anthocyanin extractability (EA)	Very Low	Low	Very Low	Very Low	0.05
Seed colorization (SC)	High	Medium	Medium	High	0.15
pH	Very High	Very High	Very High	High	0.20
Skin phenolics (Dpell)	Low	Low	Medium	Medium	0.10

Since, the FMCDM interface system has been developed; the result of the system score for the wine quality grade is calculated. It is a numerical value which corresponds to a fuzzy set. The output parameter of the FMCDM system is ranging from zero to one. The numeric value of the output is the wine quality which can belong among five levels: very poor (0 - 0.26), poor

(0.2 - 0.48), Good (0.44 - 0.69), Very Good (0.65 - 0.91) and Excellent (0.82 - 1). Figure 4.6 shows the membership function which was based on the experience and knowledge of experts.

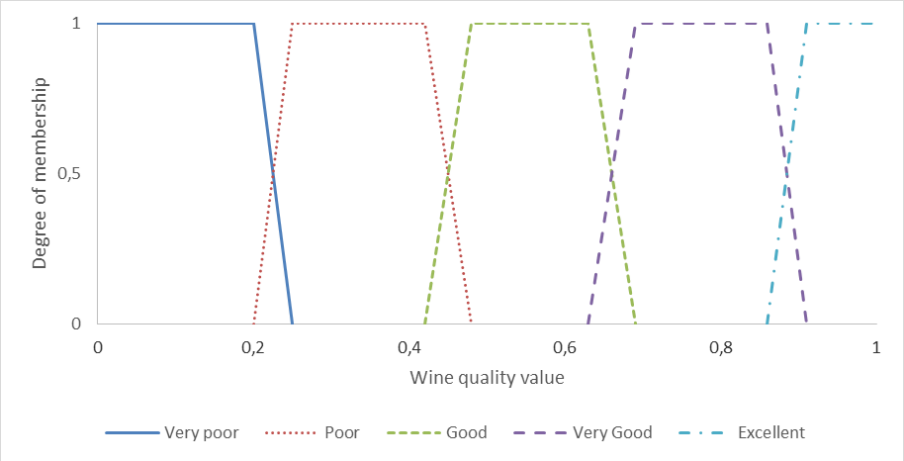

Figure 4.6: Output membership function

4.6.1 Assessing agreement between the two methods

Generally, in order to compare two ranking techniques the most usual methods are either correlation coefficients or through statistical methods (Bland et al., 1986; Petrokofsky et al., 2012). In fuzzy interface systems (FIS), alternative approaches based on simple calculations have been used in order to show the agreement between FIS results and the human expert evaluation. In this paper, the expert evaluation of each vineyard for vintages 2012, 2013 and 2014 was compared with the fuzzy evaluation as in Mazloumzadeh et al (2010) that used FIS to characterize the tree quality in date palm trees, Alavi (2013), that used FIS for grading of Mozafati dates and Tagarakis et al. (2014), that applied a FIS to model the grape quality in a vineyard. In these studies, the overall agreement between FIS and human experts was more than 87%, 90% and 80% respectively. In addition, an agreement between the descending ranking order of the FMCDM system and the established tasting

panel was carried out as the objective of this paper was to compare the ranking lists provided from two methods. The second percentage agreement was calculated using a function formulated as shown in Equation 2.

$$\text{Agreement} = \left[1 - \frac{(R_{FMCDM} - R_{Taste})}{N_{vin}}\right] \cdot 100 \quad (2)$$

Where R_{FMCDM} is the rank of the vineyard in the FMCDM ranking table, R_{Taste} is the rank of the vineyard in the ranking table by the tasting panel and N_{vin} is the total number of vineyards.

4.6.2 Wine sensory analysis

The sensory data of the 13 wine samples from the 11 panellists for the years of the experiment are presented in Table 4.22 (Detailed results data are presented at the `Apprentices` section). The maximum scoring a wine sample could receive is 21, while 0 is the lowest. The highest scoring received in our tastings was 15.3 (Vin 2, 2012) and the lowest was 5.21 (Vin 6, 2013). The evaluation score the wines received from the tasting panel was relatively low with the majority of wines receiving scores between 10 and 14 (59.0% of the wines tasted). Only five (5) wines obtained scores above 14 (12.9%), while 23.1% of the wines were rated below 10. Despite the training of the tasting panel and the scoring criteria discussed and established, tasters evaluated the samples in comparison to commercial wines. It is generally agreed that the quality of the commercial wines is higher, since oenologists usually follow different winemaking protocols (acidity correction, commercial tannin addition, use of oak alternatives etc.). Since the tasting panel was consisted of winemakers/viticulturists of Nemea region, their experience regarding the

typicity of Agiorgitiko wine resulted to lower quality ratings. Disregarding this parameter, the sensory evaluation data highlighted the quality differences among the wine samples. The performed analysis of variance (ANOVA) showed no significant differences between the replicates supporting the reliability of the tasting panel.

Table 4.22: Mean scoring of the individual attributes by tasting panel using the modified Davis scorecard for vintage 2012 (top), 2013 (middle) and 2013 (bottom), after four sensory evaluations.

Vintage 2012									
	Appear	**Aroma**	**Acidity**	**Balance**	**Develop.**	**Finish**	**Flavour**	**Overall**	**Final score***
Vin. 1	1.47	2.94	0.97	1.25	1.78	1.28	1.89	1.67	**13.25±0.45**
Vin. 2	1.42	3.42	0.94	1.61	1.92	1.67	2.25	2.08	**15.30±0.79**
Vin. 3	0.86	2.33	0.86	1.08	1.52	1.11	1.78	1.33	**10.87±1.25**
Vin. 4	1.00	2.28	0.83	1.22	1.42	1.03	1.56	1.44	**10.78±0.51**
Vin. 5	1.25	2.22	0.69	0.81	1.19	0.81	1.39	1.17	**9.53±0.51**
Vin. 6	1.08	2.42	0.83	1.08	1.50	1.28	1.72	1.42	**11.33±1.00**
Vin. 7	1.53	2.58	0.83	1.31	1.67	1.25	1.89	1.53	**12.58±0.31**
Vin. 8	0.83	2.11	0.86	0.97	1.31	1.00	1.42	1.28	**9.78±1.24**
Vin. 9	0.81	1.53	0.78	0.81	1.08	0.92	1.14	0.92	**7.97±0.90**
Vin. 10	1.47	2.83	0.92	1.39	1.72	1.39	1.92	1.64	**13.28±0.87**
Vin. 11	1.67	3.17	0.89	1.50	1.78	1.39	2.00	1.89	**14.28±0.90**
Vin. 12	1.44	2.56	0.61	0.64	1.44	1.06	1.61	1.28	**10.64±0.55**
Vin. 13	1.36	2.94	0.83	1.17	1.58	1.14	1.72	1.58	**12.33±0.75**
Vintage 2013									
	Appear	**Aroma**	**Acidity**	**Balance**	**Develop.**	**Finish**	**Flavour**	**Overall**	**Final score***
Vin. 1	1.68	2.18	0.87	1.34	1.61	1.26	1.63	1.43	**11.98±1.07**
Vin. 2	1.66	2.46	0.95	1.36	1.81	1.52	1.78	1.67	**13.22±1.05**
Vin. 3	1.45	2.89	0.90	1.40	1.74	1.48	2.11	1.81	**13.77±1.10**
Vin. 4	1.01	2.20	0.89	1.18	1.66	1.21	1.79	1.55	**11.49±0.49**
Vin. 5	1.44	1.97	0.92	1.42	1.66	1.29	1.68	1.50	**11.87±0.97**
Vin. 6	1.66	0.65	0.47	0.42	0.60	0.44	0.55	0.42	**5.21±0.88**
Vin. 7	1.42	2.59	0.92	1.31	1.65	1.36	1.78	1.69	**12.71±1.46**
Vin. 8	1.10	1.83	0.50	0.66	1.23	0.87	1.29	1.13	**8.61±0.78**
Vin. 9	0.98	0.76	0.63	0.53	0.79	0.55	0.60	0.56	**5.40±0.70**
Vin. 10	1.65	2.60	0.87	1.24	1.71	1.29	1.79	1.64	**12.80±0.22**
Vin. 11	1.34	2.13	0.84	1.37	1.71	1.34	1.84	1.52	**12.10±0.80**
Vin. 12	1.37	2.10	0.48	0.61	1.29	0.81	1.27	0.97	**8.89±0.54**
Vin. 13	0.90	1.71	0.87	0.98	1.21	1.00	1.34	1.17	**9.16±0.69**
Vintage 2014									
	Appear	**Aroma**	**Acidity**	**Balance**	**Develop.**	**Finish**	**Flavour**	**Overall**	**Final score***
Vin. 1	1.92	2.72	0.92	1.48	1.97	1.55	2.02	2.13	**14.70±0.25**
Vin. 2	1.72	2.61	0.83	1.06	1.67	1.28	1.68	1.56	**12.41±0.27**
Vin. 3	1.36	3.05	0.87	1.49	1.99	1.50	2.02	1.93	**14.22±0.24**
Vin. 4	1.50	1.96	0.55	0.97	1.31	1.09	1.54	1.30	**10.21±0.18**
Vin. 5	1.38	1.50	0.49	0.73	1.12	0.86	1.23	0.83	**8.15±0.20**
Vin. 6	1.76	3.00	0.82	1.32	1.68	1.13	1.75	1.63	**13.08±0.28**
Vin. 7	1.77	2.63	0.84	1.23	1.65	1.28	1.71	1.77	**12.87±0.22**
Vin. 8	1.37	2.96	0.87	1.52	1.78	1.29	1.80	1.96	**13.56±0.29**
Vin. 9	1.61	1.76	0.82	1.56	1.48	1.08	1.36	1.45	**10.71±0.18**
Vin. 10	1.92	3.04	0.97	1.53	2.02	1.53	2.11	2.12	**15.24±0.28**
Vin. 11	1.78	2.73	0.72	1.24	1.79	1.21	1.74	1.73	**12.95±0.20**
Vin. 12	1.29	1.74	0.34	0.57	0.92	0.73	1.21	0.94	**7.75±0.20**
Vin. 13	1.87	2.86	0.79	1.36	1.91	1.29	2.01	1.82	**13.91±0.32**

*Final scores: the sum of scores of each attribute, (±) standard deviation of each individual tasting mean values ($p<0.05$).

Differences in weather conditions were observed among the three years of the experiment. The growing season of 2012 was characterized by elevated temperatures from flowering until harvest in comparison to 2013 and 2014 (Figure 4.1). During harvest, heavy rainfall occurred resulting to heavy botrytis load, and reduced grape quality. Even though grape sugar maturity was reached, phenolic maturity was not; resulting to unbalanced wines compared to vintage 2013 and 2014. Unlike the growing season of 2012 and 2014, 2013 was a cool season with low average temperatures during vegetative growth, slow maturation, lack of rainfall during harvest and minimum botrytis infection. Due to the weather conditions sugar maturity coincided with phenolic maturity. Finally, growing season 2014 was characterized by increased rainfall during flowering and fruit set, causing extended downy mildew infection to the region. From flowering to harvest, temperatures remained low, giving place to intense rainfall during harvest resulting in extended botrytis infection.

Climatic conditions of the vintage influence the grape composition significantly. The `terroir` effect that refers to a rather small area with similar soil and microclimate conditions, affects food products distinctive quality (Barham, 2003), like wine metabolites (Brescia et al., 2002; van Leeuwen et al., 2004; Pereira et al., 2007). Each vineyard site has its own unique terroir, which is reflected in the corresponding wines more or less consistently from year to year, and to some degree, regardless of variations in methods of viticulture and winemaking (Wilson, 1998). Our results suggest that vintage had a great effect on grape composition but this effect is not equally evident among all vineyards (Perreira et al., 2006 and Rouiller-Gal et al., 2014).

4.6.3 FMCDM Grape-Wine model

The measurements of the selected parameters to be used in the FMCDM system were executed in representative berry samples from each vineyard for the three vintages and are presented in Table 4.23.

Utilizing the FMCDM system, the level of wine quality for each product was calculated. After fuzzification process, inference rules evaluation, aggregation and defuzzification, the output of each vineyard from the FMCDM system is in essence the crisp output value which ranges from 0 to 1 and it is interpreted through membership degrees of the different fuzzy sets (Figure 4.5). The results of the FMCDM system of each vineyard for the three vintages respectively, are presented in Table 4.24. In addition, the output score of the FMCDM system was normalized in the same range as the sensory evaluation results (0 to 20) and the results are presented in Table 4.24. The evaluation of each vineyard and the percentage of the agreement between the FMCDM output and the expert opinion are also included in Table 4.24. Furthermore, the descending ranking order of the 13 vineyards of the experiment is presented in Table 4.24, where the results of the FMCDM system are given in comparison to the tasting evaluation panel results. Using the descending ranking order by the two systems, the percentage agreement of the two methods was calculated (Equation 2) and it is depicted in Table 4.24.

Table 4.23: Grape quality inputs: optical density, total soluble solids, pH, berry volume, seed colorization, anthocyanin extractability, skin tannins and botrytis infection.

		Vineyard												
Vintage 2012	**Unit**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Berry volume	mL	188.3	183.3	230.0	181.7	225	221.7	228.3	190.0	195.0	221.7	140.0	238.3	148.3
Total Soluble Solids	°Baume	14.1	14.4	13.0	12.8	12,8	13.0	13.9	12.6	12.7	13.1	12.9	11.9	12.0
Botrytis infection*	-	0	0	0	0	1	0	0	1	0	0	0	1	1
Optical density (OD 520) (value x100)	-	21.7	15.6	18.7	16.8	19,2	15.4	25.9	16.1	17.2	14.7	20.4	29.0	28.3
Anthocyanin Extractability (EA)	%	44.7	35.8	22.4	31.3	17,1	35.0	28.5	20.6	40.0	28.1	30.5	38.9	35.1
pH	-	3.74	3.86	3.84	3.77	3,24	3.73	3.52	3.89	4.18	3.70	3.67	3.31	3.41
Seed maturity**	-	0	1	1	1	0	0	1	2	2	1	0	2	2
Skin phenolics (Dpell)	mg/L	6.85	7.57	7.92	9.41	10,44	10.29	8.68	10.19	4.94	12.11	8.77	11.09	8.30
		Vineyard												
Vintage 2013	**Unit**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Berry volume	mL	202.5	142.5	172.5	230.0	205	160.0	155.0	165.0	190.0	177.5	190.0	200.0	217.5
Total Soluble Solids	°Baume	14.1	13.4	12.5	13.6	14,1	14.1	13.4	12.1	13.1	13.1	14.2	12.3	12.6
Botrytis infection*	%	0	0	0	0	0	0	0	0	0	0	0	0	0
Optical density (OD 520) (value x100)	-	19.8	21.9	18.8	12.2	17,9	20.7	16.9	17.6	17.0	19.9	15.6	14.1	9.8
Anthocyanin Extractability (EA)	%	28.4	31.7	37.0	27.4	45.5	38.1	29.4	52.0	28.5	20.0	24.6	42.0	31.9
pH	-	3.67	3.67	3.63	3.74	3.54	3.53	3.67	3.56	3.89	3.56	3.87	3.21	3.40
Seed maturity**	-	0	1	1	1	0	0	1	2	2	1	0	2	2
Skin phenolics (Dpell)	mg/L	10.06	12.14	10.06	7.45	7.73	10.13	10.78	6.99	9.33	10.37	9.61	9.38	7.00
		Vineyard												
Vintage 2014	**Unit**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Berry volume	mL	197.50	225.00	177.5	225.0	237.5	170.0	180.0	167.5	155.0	182.5	210.0	202.5	197.5
Total Soluble Solids	°Baume	14.5	12.9	13.3	14.0	11.0	13.1	13.0	13.1	12.2	12.8	14.0	12.0	13.8
Botrytis infection*	%	0	0	1	0	0	1	1	2	2	1	0	0	0
Optical density (OD 520) (value x100)	-	18.7	21.1	17.4	15.4	17.9	24.7	24.1	15.2	14.9	25.4	19.6	13.4	21.4
Anthocyanin Extractability (EA)	%	40.2	24.1	28.1	45.3	43.6	24.8	48.9	36.4	51.6	40.6	21.3	37.4	43.6
pH	-	3.65	3.49	3.64	3.41	3.17	3.43	3.43	3.52	3.42	3.40	3.69	3.28	3.39
Seed maturity**	-	0	2	1	1	2	2	2	2	2	0	1	2	1
Skin phenolics (Dpell)	mg/L	11.12	12.3	6.80	8.21	6.34	13.44	12.13	5.70	5.46	12.71	9.79	5.23	13.57

Values are the means of triplicate determination ($p<0.05$). *Botrytis infection: 0 = no infection, 1= < 5% infection, 2 = > 5%infection.

**Seed maturity: according to Fredes et al. (2010). 0 = brown colorization, 1 = slight green colorization, 2 = 50% brown, 50% green colorization

4.6.4 Comparison of the two methods

The results presented in Table 4.24 showed that FMCDM system is able to model human expertise successfully. For example, Vin 4 in 2012 was classified as “good” by the experts whereas FMCDM system placed it as 85% in the “good” and 15% in the “Very Good”. In this case, there is an agreement between FMCDM results and expert evaluation of 85%. Similarly, the same vineyard was classified as “Very Good” by the experts for vintage 2013 whereas FMCDM placed it as “Very Good”. Therefore, in this case the evaluation agreement was 100%.

In addition, from the results presented in Table 5, it can be concluded that a relationship between the FMCDM system ranking and the wine tasting panel ranking could be established. In most cases the relationship was very strong and was mainly observed among the highest and lowest ranked vineyards respectively. Indeed, the evaluations of wine quality carried out by the two methods were comparable. So, the proposed FMCDM system is effective in the sense that it is accurate in identification of wine quality because it is based on literature and the knowledge of oenologist experts on grapes attributes.

Furthermore, with the FMCDM system, the wine quality can move from a linguistic description to a quantifiable representation without further computational overhead; while a grade figure is created that correspond to the wine quality. In some cases the results of the FMCDM system failed to agree with the preference of the tasting panel, with Vin 8 during vintage 2014 and Vineyard 3 during vintage of 2013, showing the greatest disagreement (Table 4.24).

Table 4.24: Ranking of the vineyard in descending order, according to the tasting panel in comparison to the ranking results of the FMCDM system, for vintage 2012 (top), 2013 (middle) and 2013 (bottom)

2012								
Vineyard	**Output score**	**Output score normalized as the sensory evaluation**	**Fuzzy evaluation**	**Expert evaluation**	**Agreement of evaluation (%)**	**Ranking by Tasting panel**	**Ranking by FMCDM system**	**Agreement of ranking (%)**
1	0.781	15.09	100% in VG	VG	100	4	3	92.3
2	0.872	17.13	76% in VG and 24% in E	VG	76	1	1	100
3	0.475	8.22	8.3% in P and 91.7 % in G	G	91.7	8	9	92.3
4	0.639	11.91	85% in G and 15% in VG	G	85	9	7	84.6
5	0.350	5.41	100% in P	P	100	12	12	100
6	0.454	7.75	43% in P and 57% in G	G	57	7	10	76.9
7	0.852	16.67	100% in VG	VG	100	5	2	76.9
8	0.555	10.02	100% in G	G	100	11	8	76.9
9	0.339	5.18	100% in P	P	100	13	13	100
10	0.776	14.97	100% in VG	VG	100	3	4	92.3
11	0.761	14.63	100% in VG	VG	100	2	5	76.9
12	0.353	5.49	100% in P	P	100	10	11	92.3
13	0.670	12.61	33% in G and 67% in VG	VG	67	6	6	100
2013								
Vineyard	**Output score**	**Output score normalized as the sensory evaluation**	**Fuzzy evaluation**	**Expert evaluation**	**Agreement of evaluation (%)**	**Ranking by Tasting panel**	**Ranking by FMCDM system**	**Agreement of ranking (%)**
1	0.777	15.00	100% in VG	VG	100	3	4	92.3
2	0.937	18.59	100% in E	VG	0	2	1	92.3
3	0.592	10.84	100% in G	VG	0	1	8	46.1
4	0.771	14.86	100% in VG	VG	100	7	5	84.6
5	0.781	15.09	100% in VG	VG	100	8	3	61.5
6	0.171	1.39	100% in P	P	100	13	13	100
7	0.799	15.50	100% in VG	VG	100	5	2	76.9
8	0.553	9.97	100% in G	G	100	11	9	84.6
9	0.515	9.12	100% in G	P	0	12	10	84.6
10	0.741	14.19	100% in VG	VG	100	4	6	84.6
11	0.713	13.56	100% in VG	VG	100	6	7	92.3
12	0.485	8.45	100% in G	G	100	10	12	84.6
13	0.511	9.03	100% in G	G	100	9	11	84.6
2014								
Vineyard	**Output score**	**Output score normalized as the sensory evaluation**	**Fuzzy evaluation**	**Expert evaluation**	**Agreement of evaluation (%)**	**Ranking by Tasting panel**	**Ranking by FMCDM system**	**Agreement of ranking (%)**
1	0.714	13.59	100% in VG	VG	100	1	2	92.3
2	0.804	15.61	100% in VG	VG	100	10	6	69.2
3	0.684	12.92	10% in G and 90% in VG	VG	90	3	7	69.2
4	0.500	8.79	100% in G	G	100	13	10	76.9
5	0.345	5.32	100% in P	P	100	6	1	61.5
6	0.795	15.40	100% in VG	VG	100	8	3	61.5
7	0.786	15.20	100% in VG	G	0	11	13	84.6
8	0.564	10.23	100% in G	VG	0	2	12	23.1
9	0.564	10.23	100% in G	G	100	7	8	92.3
10	0.734	14.04	100% in VG	VG	100	9	9	100
11	0.500	8.79	100% in G	G	100	4	4	100
12	0.604	11.12	100% in G	P	0	12	11	92.3
13	0.654	12.24	60% in G and 40% in VG	VG	40	5	5	100

Very Poor: VP, Poor: P, Good: G, Very Good: VG, Excellent: E

More specifically, out of the thirty nine (39) vineyards for both seasons, eighteen (18) vineyards showed high degree of agreement between the ranking of the FMCDM system ranking and the sensory panel (between 92.3% and 100%), sixteen (16) vineyards showed agreement above 76.9% and five (5) vineyards agreed poorly (Vin 5 and Vin 3 in vintage 2013; Vin 5, Vin 6 and Vin 8 in vintage 2014). This mismatch could be attributed to sensory parameters that could not be included to the current model system (e.g. astringency, characteristic volatile compounds). Furthermore, by examining the output score after normalization in the same scale for the sensory and FMCDM systems, it is observed that disparities between the two methods appear. However, these differences are quite normal and they are attributed to the different nature of the variables used in each method. Indeed, while `aroma and bouquet` and `flavour` are parameters that can be easily perceived by the tasters it is unfeasible to be included to the current FMCDM system as it refers to grape parameter.

Furthermore, the attribute `aroma and bouquet`, was measured by a five point-scale (5), while the rest attributes by a maximum of three point-scale (3). The Davis scoring sheet has been designed to emphasize on aroma since it is consider the clearest indicator of the grape`s varietal character (Winiarsky et al., 1996). Very few published studies exist regarding the aromatic profile of Agiorgitiko (Koussissi et al., 2002; 2003) and its varietal character is an important parameter that should be highlighted. In fact, important aspects of wine are thought to be its varietal distinctiveness and increasingly winemakers pursuit for `distinctive' wines and not for 'better' wines as they used to in the past. These specific organoleptic parameters and properties should be further

studied, evaluated and included in updated version of the scoring sheet. In addition, the samples that received higher `Aroma and bouquet` score received also higher `Flavour` score indicating the strong connection between these two parameters. Moreover the attribute `Balance` is difficult to be defined by the tasters due to its vague definition and perception, while it is practically unfeasible to include it into the FMCDM system. Despite the existing difficulties in measuring and perceiving the above parameters, their significant contribution on the overall wine quality is generally accepted.

As seen on Table 4.24, Vin 8 (vintage 2014) and Vin 3 (vintage 2013) presented the first and second highest disagreement between the tasting panel and the FMCDM ranking (23.1% and 46.1 respectively). Vin 3 (vintage 2013) received the highest scoring by the panellists for the attributes `Aroma and bouquet`, `Flavour` and `Balance`, (2.89, 2.11 and 1.40 respectively), while Vin 8 (vintage 2014), received high scoring in `Aroma and bouquet` and mouth-feel attributes (Balance, Development, Overall). These parameters were not taken into consideration when designing the FMCDM system due to reasons explained previously.

On the other hand; Vin 5 (vintage 2013) and Vin 5 (vintage 2014) received higher scores by the panel than by the FMCDM system evaluation. As showed in Tables 4.20 and 4.23, both vineyards, received lower FMCDM scores of parameters related to phenolic composition (EA, BV, Dpell), which are represented in the tasting score only by the attribute `Finish`. Indeed attributes as `astringency`, `tannin quality`, `texture` are not included in the Davis tasting sheet, failing to describe the complexity of current wines (Winiarsky, 1996).

Finally, Vin 6 (vintage 2014) also presented disagreement between the tasting panel and the FMCDM ranking, combining the conditions discussed above. The vineyard, received high scoring by the tasting panel for the attribute `Aroma and bouquet` but received low scoring in attributes related to mouthfeel perception. In contrast the ranking according to FMCDM, was high since most of the parameters presented values ranging from `Very good` to `Excellent`.

4.6.5 Conclusions

Wine quality prediction depends on many grape parameters, connected in a non-linear and complex matrix. In the present work, a FMCDM system was developed for the estimation of wine quality by synthesizing a number of important grape parameters. The FMCDM system was based on the extraction of the experience and knowledge of oenologist experts and literature for the selection of the variables as well as for the evaluation of the importance of each parameter. A set of linguistic variables and rules were built to present the relationship between the grape and wine quality. A comparison between the wine tasting and the FMCDM system was carried out and the results showed that the FMCDM system ranked the wines in a similar manner with the wine experts. The exceptions observed between the two methods are related to parameters that even though are important for wine quality is unfeasible to include them into the FMCDM system (e.g. characteristic volatile compounds) and/or attributes not adequately represented in the tasting sheet (e.g. astringency).

Despite these weaknesses, it is possible to estimate wine quality from easily measurable grape parameters applied in a FMCDM system. The proposed tool is simple, quick and economical, able to objectively evaluate grape composition and correlate it with the respective wine quality. It could be a starting point for the design of more specific models according to the requirements of the wineries in other wine producing regions and for other grape varieties since estimation of wine quality remains a priority.

General conclusions.

5. General conclusions.

The aim of this study was to evaluate the grape and wine quality parameters of Agiorgitiko grown in Nemea wine region. For that purpose selected vineyards of the region were studied for three consecutive years and extensive chemical analyses were performed. The key chemical analyses findings could be summarized:

- Grape spectrophotometric analyses revealed the importance of skin and seed content and ratio to the total tannin content, suggesting that could be used as marker of grape quality. In contrast unlike common belief, berry weight and volume did not affect grape anthocyanin and phenolic content.
- Comparing Agiorgitiko with other Greek and international grape varieties, it is a variety rich in anthocyanins and due to the low mean degree of polymerization (mDP), percentage of galloylation (%G); in combination with the abundance of (-)-epigallocatechin (EGC) subunits, it is suggested that Agiorgitiko is a low astringent grape variety.
- Grape and wine proanthocyanidin analyses showed that skin PAs might have an important role in the final wine PA composition and content, unlike seed PAs which seem to be of minor importance. However, a possible connection between grape and the corresponding wine PA composition was not observed.
- Concerning nitrogen and amino acid content, Agiorgitiko is a cultivar with reduced yeast assimilable nitrogen (YAN) and ammonia nitrogen, in most cases marginally adequate for successful alcoholic fermentation. Most vineyard characteristics evaluated in our study (e.g.

rootstock, vine density, maturity) had a minor impact and only on individual AAs.

- A strong vintage effect was evident in most analyses performed. More specifically, increased water supply was positively correlated to grape anthocyanin and phenolic content, grape skin mean degree of polymerization (mDP) but negatively correlated to the skin percentage of prodelphinidins (%P) and grape amino acid content. Ambient temperature during ripening was positively correlated only to amino acid content.
- From the vineyard characteristics recorded, only altitude had a positive effect on seed proanthocyanidins and negative on individual amino acid content.

An important aim of this study was to establish relations between the grape chemical composition and the quality of the produced wine. We established a fuzzy logic multi criteria decision making (FMCDM) system, for the estimation of wine quality by synthesizing a number of important grape parameters: berry volume (BV), total soluble solids (TSS), Botrytis infection, Optical Density (OD 520), anthocyanin extractability (EA), seed colorization (SC), pH and skin phenolic content (Dpell). Professional tasting panel evaluated the wine samples, a comparison between the wine tasting and the FMCDM system was carried out and the results showed that the FMCDM system ranked the wines in a similar manner with the wine experts. Exceptions were observed between the two methods but despite these weaknesses, it was possible to estimate wine quality from easily measurable grape parameters applied in a FMCDM system. Further

improvements are required since some parameters of wine quality are unfeasible to be included into the FMCDM system (e.g. characteristic volatile compounds). Furthermore, modifications are required in the tasting sheet since some attributes are not adequately represented in the tasting sheet (e.g. astringency) in conjunction with update on the score-scaling of some attributes (e.g. `Aroma and bouquet`), would more accurately represent wine quality.

Finally, in this study we investigated the grape and wine composition of vineyards from different sub-regions of Nemea. More specifically, grapes were sourced from five (5) different sub-regions of Nemea: Koutsi, Nemea Valley, Tsintaria (west slopes of Koutsi), Ancient Nemea and Asprokampos. The results of most grape and chemical analyses failed to categorize the vineyards according to sub-regions and differences were observed only among individual vineyards. However, as discussed, grape and wine chemical parameters were affected by climatic conditions (e.g. water supply), which are crucial parameters of `terroir`. As an example, the sub region of Asprokampos is established in altitude above 700 m; more likely related to lower ambient temperature, higher rainfall and delayed maturity against lower altitude sub regions. In this study, meteorological data were available only from the meteorological station established in the Valley of Nemea and not from the individual sub regions. Therefore, correlations among individual sub regions climatic conditions and grape and wine chemical attributes could not be accurately established. In addition this study was focused in grape and wine chemical analyses and their correlations without taking into account viticultural practices and

techniques such as yield, pruning, tillage, disease management etc. Such parameters are critical for grape and wine quality able to alter comparison among vineyards established to different sub regions.

Future work.

- Modification of the FMCDM system optimizing the representation of wine quality.
- Application of the FMCDM system in `real time` winemaking conditions.
- Application of the FMCDM system mapping `distinctive` or `problematic` vineyards.
- Implementation of the FMCDM system in the appellation system of A.O.C Nemea.
- Adaptation of the FMCDM system to other Greek and international grape varieties (e.g. Xinomavro).
- Further research on grape and wine chemical components that differentiate individual wine sub regions of Nemea.

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Appendices.

Vineyard selection and grape sampling methodology.

1) In collaboration with the wineries collaborating to our research, we were designated candidate vineyards with variability in grape yield, botrytis susceptibility, grape quality and vineyard health condition according to their experience.
2) The fourteen vineyards participating to the research were selected, located in different sub-regions of Nemea (Koutsi, Nemea Valley, Tsintaria, Ancient Nemea, Asprokampos).
3) Approximately 100 vines were marked in each selected vineyard.
4) Harvest date was designated according to weather conditions and grape maturity as indicated by sugar content (^{o}Baume). No specific maturity level was targeted apart from being in the range between 11^{o} to 14^{o} Baume.
5) On harvest day, approximately 50 kgs of grapes were picked and from each bunch two-three berries were randomly selected. Part of the collected berries was used for grape measurements, while a sub-sample of 300 berries was randomly selected and stored at -18 ^{o}C for further analyses.

Table A1: Calibration curves for quantification of grape and wine proanthocyanidins, determined by HPLC. `e`: extension units with bound with phloroglucinol, `t`: terminal units. `x`: area of each pick quantified.

Calibration curves (µmol)	
EGCe	y=0.0239x
Ce	y=0.0027x
ECe	y=0.0047x
EGCt	y=0.0235x
Ct	y=0.0026x
ECt	y=0.0045x
ECGe	y=0.00017x
EGCGt	y=0.0028x
ECGt	y=0.0017x

C:(+)-catechin, EC:(-)-epicatechin, ECG:(-)-epicatechin-3-O-gallate subunits, EGC:(+)-gallocatechin, (-)-epigallocatechin, EGCG:(-)-epigallocatechin 3-O-gallate.

Table A2: Calibration curves for quantification of grape and wine amino acids.

Calibration curves (mg/lt)	
L-aspartic acid	y=0.5079x+0.0133
L-glutamic acid	y=0.4339x-0.0028
L-asparagine	y=0.6774x+0.0665
L-serine	y=0.8485x+0.0004
L-glutamine	y=0.5919-0.0052
L-histidine	y=0.3054-0.0093
L-glycime	y=0.8014+0.0431
L-threonine	y=0.7361-0.0044
L-alanine	y=0.9769+0.0755
L-arginine	y=0.4742+0.0480
L-tyrosine	y=0.4230-0.0021
L-valine	y=0.8499-0.0109
L-tryptophan	y=0.4155+0.0030
L-leucine	y=0.6967-0.0007
L-lysine	y=0.1130+0.0035
L-hydroxyproline	y=3.0202+0.0567
L-Proline	y=0.9690+0.0994

Table A3: Wine sensory evaluation results of the four tasting sessions (vintage 2012). Final scores are the sum of scores of each attribute.

Vintage 2012: Tasting #1									
	Appear	Aroma	Acidity	Balance	Develop	Finish	Flavor	Overall	Final score
Vin. 1	1.44±0.53	2.67±0.50	1.00±0.00	1.22±0.63	1.89±0.60	1.44±0.50	1.67±0.50	1.89±0.78	**13.22**
Vin. 2	1.44±0.53	3.11±0.93	1.00±0.00	1.89±0.32	2.11±0.33	1.89±0.46	2.22±0.44	2.33±0.50	**16.00**
Vin. 3	0.89±0.33	2.33±0.70	0.89±0.33	1.44±0.49	1.89±0.33	1.22±0.40	1.89±0.33	1.67±0.50	**12.22**
Vin. 4	0.89±0.60	1.78±0.83	0.78±0.44	1.22±0.42	1.44±0.53	1.11±0.54	1.56±0.53	1.67±0.50	**10.44**
Vin. 5	1.33±0.50	2.33±0.71	0.44±0.53	1.00±0.47	1.56±0.53	0.89±0.54	1.22±0.44	1.33±0.50	**10.11**
Vin. 6	1.22±0.67	1.78±0.97	0.78±0.44	1.33±0.67	1.56±0.88	1.56±0.53	1.56±0.53	1.44±0.73	**11.22**
Vin. 7	1.56±0.53	2.44±0.88	0.78±0.44	1.56±0.50	1.78±0.67	1.33±0.47	1.78±0.67	1.67±0.50	**12.89**
Vin. 8	0.89±0.33	1.78±0.83	0.78±0.44	1.22±0.63	1.56±0.53	1.33±0.47	1.22±0.44	1.44±0.73	**10.22**
Vin. 9	0.89±0.33	1.00±0.87	0.78±0.44	0.89±0.32	0.78±0.44	1.11±0.54	0.89±0.60	0.78±0.44	**7.11**
Vin. 10	1.56±0.53	2.67±0.87	0.89±0.33	1.56±0.68	1.89±0.60	1.67±0.52	1.78±0.83	1.89±0.60	**13.89**
Vin. 11	1.78±0.44	2.89±0.60	0.89±0.33	1.67±0.47	2.00±0.50	2.00±0.00	1.89±0.60	2.00±0.71	**15.11**
Vin. 12	1.44±0.53	2.33±0.70	0.44±0.53	0.67±0.67	1.67±0.50	1.33±0.65	1.22±0.44	1.44±0.73	**10.56**
Vin. 13	1.33±0.50	2.33±0.70	0.67±0.50	1.33±0.47	1.67±0.50	1.44±0.50	1.44±0.73	1.67±0.50	**11.89**

Vintage 2012: Tasting #2									
	Appear	Aroma	Acidity	Balance	Develop	Finish	Flavor	Overall	Final score
Vin. 1	1.44±0.53	3.00±1.11	1.00±0.00	1.11±0.60	1.67±0.50	1.33±0.50	1.78±0.67	1.56±0.53	**12.89**
Vin. 2	1.44±0.53	3.67±0.70	0.89±0.33	1.56±0.53	1.78±0.67	1.56±0.53	2.11±0.33	2.00±0.71	**15.00**
Vin. 3	0.78±0.44	2.11±0.93	0.89±0.33	0.89±0.30	1.22±0.44	1.22±0.44	1.78±0.44	1.22±0.44	**10.11**
Vin. 4	1.11±0.33	2.67±1.00	0.78±0.44	0.89±0.53	1.33±0.50	1.11±0.78	1.67±0.50	1.33±0.50	**10.89**
Vin. 5	1.00±0.00	2.11±0.78	0.78±0.44	0.89±0.65	1.11±0.33	0.67±0.50	1.33±0.50	1.00±0.00	**8.89**
Vin. 6	1.00±0.00	2.67±1.22	0.56±0.53	0.67±0.47	1.33±0.71	0.89±0.60	1.67±0.87	1.33±0.71	**10.11**
Vin. 7	1.56±0.73	2.89±0.93	0.67±0.50	1.22±0.77	1.67±0.50	1.33±0.50	2.00±0.50	1.44±0.53	**12.78**
Vin. 8	0.78±0.44	1.89±0.93	0.78±0.44	0.67±0.47	1.11±0.60	0.89±0.33	1.56±0.53	1.11±0.33	**8.78**
Vin. 9	0.78±0.67	2.22±0.93	0.67±0.50	0.89±0.65	1.56±0.53	0.67±0.50	1.11±0.60	1.11±0.33	**9.00**
Vin. 10	1.56±0.53	2.67±1.22	0.89±0.33	1.11±0.65	1.67±0.50	1.22±0.44	1.67±0.70	1.33±0.71	**12.11**
Vin. 11	1.56±0.53	3.56±0.53	0.78±0.44	1.11±0.54	1.56±0.53	1.22±0.44	2.00±0.00	1.67±0.50	**13.44**
Vin. 12	1.44±0.53	2.22±0.83	0.56±0.53	0.56±0.52	1.22±0.44	1.00±0.00	1.67±0.50	1.22±0.44	**9.89**
Vin. 13	1.56±0.53	3.33±0.87	0.78±0.44	1.00±0.75	1.44±0.73	0.89±0.60	1.67±0.50	1.44±0.53	**12.11**

Vintage 2012: Tasting #3									
	Appear	Aroma	Acidity	Balance	Develop	Finish	Flavor	Overall	Final score
Vin. 1	1.44±0.53	3.11±0.60	1.00±0.00	1.44±0.53	1.89±0.60	1.11±0.33	2.11±0.33	1.78±0.44	**13.89**
Vin. 2	1.33±0.50	3.56±0.88	1.00±0.00	1.67±0.50	2.00±0.44	1.78±0.44	2.33±0.71	2.22±0.44	**15.89**
Vin. 3	1.00±0.00	2.44±0.73	1.00±0.00	1.22±0.44	1.50±0.79	1.11±0.33	1.78±0.44	1.56±0.53	**11.61**
Vin. 4	0.78±0.44	1.78±0.97	1.00±0.00	1.44±0.73	1.56±0.50	1.00±0.00	1.44±0.73	1.33±0.71	**10.33**
Vin. 5	1.44±0.53	1.89±0.93	0.78±0.44	0.67±0.50	1.11±0.47	1.00±0.00	1.44±0.53	1.33±0.50	**9.67**
Vin. 6	1.00±0.00	2.11±0.93	1.00±0.00	1.22±0.44	1.67±0.69	1.44±0.53	1.56±0.53	1.44±0.53	**11.44**
Vin. 7	1.67±0.50	2.11±1.05	1.00±0.00	1.22±0.67	1.67±0.83	1.22±0.44	1.89±0.60	1.67±0.71	**12.44**
Vin. 8	1.00±0.50	2.22±0.67	1.00±0.00	1.22±0.44	1.56±0.75	1.11±0.33	1.67±0.71	1.56±0.53	**11.33**
Vin. 9	0.89±0.33	0.78±0.67	0.89±0.33	0.67±0.50	1.11±0.30	0.89±0.33	1.22±0.44	0.89±0.33	**7.33**
Vin. 10	1.33±0.50	2.89±0.93	1.00±0.00	1.67±0.50	1.89±0.65	1.33±0.50	2.11±0.33	1.78±0.67	**14.00**
Vin. 11	1.67±0.50	2.89±1.17	1.00±0.00	1.78±0.44	2.00±0.60	1.22±0.44	2.22±0.67	2.22±0.67	**15.00**
Vin. 12	1.33±0.50	2.44±0.88	0.78±0.44	1.00±0.87	1.44±0.52	1.00±0.50	1.78±0.67	1.33±0.50	**11.11**
Vin. 13	1.11±0.33	2.56±0.73	1.00±0.00	1.22±0.44	1.56±0.52	1.11±0.33	1.78±0.67	1.56±0.53	**11.89**

Vintage 2012: Tasting #4									
	Appear	Aroma	Acidity	Balance	Develop	Finish	Flavor	Overall	Final score
Vin. 1	1.56±0.53	3.00±1.18	0.89±0.33	1.22±0.44	1.67±0.503	1.22±0.44	2.00±0.00	1.44±0.73	**13.00**
Vin. 2	1.44±0.53	3.33±0.71	0.89±0.33	1.33±0.71	1.78±0.67	1.44±0.53	2.33±0.50	1.78±0.67	**14.33**
Vin. 3	0.78±0.44	2.44±0.88	0.67±0.50	0.78±0.44	1.44±0.73	0.89±0.33	1.67±0.50	0.89±0.60	**9.56**
Vin. 4	1.22±0.44	2.89±0.60	0.78±0.44	1.33±0.50	1.33±0.50	0.89±0.33	1.56±0.73	1.44±0.53	**11.44**
Vin. 5	1.22±0.44	2.56±0.53	0.78±0.44	0.67±0.71	1.00±0.50	0.67±0.50	1.56±0.53	1.00±0.00	**9.44**
Vin. 6	1.11±0.33	3.11±1.05	1.00±0.00	1.11±0.60	1.44±0.53	1.22±0.44	2.11±0.60	1.44±0.53	**12.56**
Vin. 7	1.33±0.50	2.88±0.83	0.89±0.33	1.22±0.44	1.56±0.53	1.11±0.60	1.89±0.60	1.33±0.50	**12.21**
Vin. 8	0.67±0.50	2.56±0.88	0.89±0.33	0.78±0.44	1.00±0.42	0.67±0.50	1.22±0.83	1.00±0.50	**8.78**
Vin. 9	0.67±0.50	2.11±1.05	0.78±0.44	0.78±0.67	0.89±0.60	1.00±0.50	1.33±0.71	0.89±0.60	**8.44**
Vin. 10	1.44±0.53	3.11±1.67	0.89±0.33	1.22±0.44	1.44±0.73	1.33±0.50	2.11±0.33	1.56±0.53	**13.11**
Vin. 11	1.67±0.50	3.33±0.87	0.89±0.33	1.44±0.53	1.56±0.73	1.11±0.33	1.89±0.60	1.67±0.50	**13.56**
Vin. 12	1.56±0.53	3.22±0.44	0.67±0.50	0.33±0.50	1.44±0.53	0.89±0.33	1.78±0.44	1.11±0.60	**11.00**
Vin. 13	1.44±0.53	3.56±1.01	0.89±0.33	1.11±0.78	1.67±0.87	1.11±0.78	2.00±0.00	1.67±0.87	**13.44**

(±) standard deviation of each individual tasting mean values (n=11).

Table A4: Wine sensory evaluation results of the four tasting sessions (vintage 2013). Final scores are the sum of scores of each attribute.

Vintage 2013: Tasting #1									
	Appear	Aroma	Acidity	Balance	Develop	Finish	Flavor	Overall	Final score
Vin. 1	1.70±0.48	2.60±1.58	0.90±0.32	1.50±0.71	1.70±0.95	1.40±0.70	2.00±0.82	1.70±0.82	**13.50**
Vin. 2	1.70±0.48	2.50±0.97	1.00±0.00	1.40±0.52	1.60±0.52	1.50±0.53	1.70±0.48	1.50±0.71	**12.90**
Vin. 3	1.50±0.53	3.20±1.23	1.00±0.00	1.40±0.52	1.80±0.42	1.50±0.53	2.10±0.57	2.00±0.82	**14.50**
Vin. 4	0.80±0.63	2.70±0.67	1.00±0.00	1.30±0.48	1.70±0.82	1.30±0.48	1.70±0.67	1.70±0.67	**12.20**
Vin. 5	1.60±0.52	2.10±0.88	1.00±0.00	1.40±0.52	1.70±0.67	1.30±0.48	1.80±0.78	1.70±0.67	**12.60**
Vin. 6	1.80±0.42	0.80±0.79	0.60±0.52	0.40±0.52	0.60±0.70	0.70±0.48	0.60±0.52	0.50±0.71	**6.00**
Vin. 7	1.40±0.52	3.00±0.82	1.00±0.00	1.60±0.52	1.90±0.32	1.50±0.53	2.10±0.32	2.10±0.32	**14.60**
Vin. 8	1.20±0.63	2.20±0.79	0.50±0.53	0.70±0.67	1.40±0.52	0.90±0.57	1.20±0.63	1.30±0.67	**9.40**
Vin. 9	0.90±0.32	0.60±1.07	0.50±0.53	0.50±0.71	0.90±0.99	0.60±0.70	0.60±0.70	0.50±0.71	**5.10**
Vin. 10	1.80±0.42	2.50±0.85	0.70±0.48	1.20±0.63	1.60±0.70	1.20±0.42	1.90±0.57	1.60±0.52	**12.50**
Vin. 11	1.20±0.63	2.10±1.10	0.80±0.42	1.30±0.67	1.80±0.63	1.10±0.57	1.70±0.67	1.50±0.85	**11.50**
Vin. 12	1.50±0.58	2.00±0.82	0.40±0.52	0.50±0.53	1.10±0.74	0.80±0.42	1.10±0.57	0.90±0.74	**8.30**
Vin. 13	0.70±0.48	1.70±1.06	0.80±0.42	0.80±0.63	1.20±0.63	1.00±0.47	1.30±0.67	1.10±0.74	**8.60**

Vintage 2013: Tasting #3									
	Appear	Aroma	Acidity	Balance	Develop	Finish	Flavor	Overall	Final score
Vin. 1	1.70±0.48	2.10±1.57	0.80±0.32	1.30±0.71	1.50±0.95	1.30±0.70	1.50±0.81	1.46±0.83	**11.66**
Vin. 2	1.70±0.48	2.90±0.97	0.90±0.00	1.50±0.52	2.10±0.52	1.70±0.53	2.10±0.48	1.84±0.71	**14.74**
Vin. 3	1.40±0.53	2.70±1.23	0.70±0.00	1.10±0.52	1.60±0.42	1.40±0.53	2.10±0.57	1.57±0.82	**12.57**
Vin. 4	0.90±0.63	2.20±0.67	0.80±0.00	1.20±0.48	1.60±0.82	1.30±0.48	1.80±0.67	1.40±0.67	**11.20**
Vin. 5	1.40±0.52	2.10±0.88	0.90±0.00	1.40±0.52	1.70±0.68	1.40±0.48	1.70±0.78	1.51±0.67	**12.11**
Vin. 6	1.60±0.42	0.70±0.79	0.50±0.52	0.60±0.52	0.70±0.70	0.50±0.48	0.60±0.51	0.74±0.71	**5.94**
Vin. 7	1.60±0.52	2.90±0.82	1.00±0.00	1.30±0.52	1.60±0.31	1.40±0.52	1.70±0.32	1.64±0.32	**13.14**
Vin. 8	1.20±0.63	2.00±0.79	0.40±0.53	0.60±0.67	1.20±0.51	0.90±0.57	1.50±0.63	1.11±0.67	**8.91**
Vin. 9	1.00±0.32	0.90±1.07	0.80±0.53	0.60±0.71	0.70±0.99	0.50±0.70	0.80±0.70	0.76±0.71	**6.06**
Vin. 10	1.70±0.42	2.80±0.85	1.00±0.48	1.10±0.63	1.70±0.70	1.30±0.42	1.70±0.57	1.61±0.52	**12.91**
Vin. 11	1.40±0.63	2.20±1.10	0.80±0.42	1.40±0.67	1.80±0.64	1.50±0.57	2.00±0.67	1.59±0.85	**12.69**
Vin. 12	1.20±0.53	2.30±0.82	0.50±0.52	0.70±0.53	1.50±0.74	0.90±0.42	1.30±0.57	1.20±0.74	**9.60**
Vin. 13	1.00±0.48	1.80±1.06	1.00±0.42	1.10±0.63	1.30±0.63	1.10±0.47	1.40±0.67	1.24±0.74	**9.94**

Vintage 2013: Tasting #2									
	Appear	Aroma	Acidity	Balance	Develop	Finish	Flavor	Overall	Final score
Vin. 1	1.56±0.53	2.11±1.45	0.78±0.44	1.11±0.60	1.56±0.88	1.22±0.67	1.44±0.73	1.22±1.09	**11.00**
Vin. 2	1.56±0.53	2.00±0.87	0.89±0.33	1.44±0.73	1.89±0.33	1.44±0.53	1.56±0.73	1.56±0.88	**12.33**
Vin. 3	1.44±0.53	3.00±0.50	1.00±0.00	1.78±0.44	1.89±0.60	1.56±0.53	2.22±0.44	2.00±0.50	**14.89**
Vin. 4	1.22±0.44	2.00±0.71	0.89±0.33	1.22±0.67	1.67±0.50	1.00±0.50	1.78±0.44	1.67±0.50	**11.44**
Vin. 5	1.22±0.44	1.56±0.88	0.89±0.33	1.44±0.53	1.67±0.50	1.11±0.33	1.22±0.83	1.33±0.71	**10.44**
Vin. 6	1.67±0.50	0.44±1.01	0.33±0.50	0.33±0.71	0.56±0.73	0.22±0.44	0.67±0.71	0.22±0.44	**4.44**
Vin. 7	1.33±0.50	2.22±1.10	0.89±0.33	1.11±0.78	1.56±1.01	1.22±0.67	1.67±0.71	1.56±0.73	**11.56**
Vin. 8	1.00±0.50	1.67±1.22	0.67±0.50	0.78±0.67	1.11±0.60	0.89±0.33	1.33±0.50	1.11±0.60	**8.56**
Vin. 9	1.00±0.50	0.89±0.39	0.67±0.50	0.44±0.53	0.89±0.33	0.56±0.53	0.78±0.67	0.67±0.50	**5.89**
Vin. 10	1.56±0.53	2.78±0.83	0.89±0.33	1.33±0.71	1.78±0.67	1.33±0.50	1.67±0.50	1.67±0.50	**13.00**
Vin. 11	1.00±0.00	2.11±0.78	0.89±0.33	1.22±0.67	1.44±0.53	1.33±0.50	1.89±0.33	1.44±0.73	**11.33**
Vin. 12	1.44±0.53	2.11±1.17	0.44±0.53	0.56±0.53	1.22±0.67	0.78±0.67	1.44±0.73	0.89±0.78	**8.89**
Vin. 13	0.89±0.73	1.44±0.73	0.78±0.44	1.00±0.50	1.11±0.33	1.00±0.50	1.33±0.50	1.00±0.50	**8.56**

Vintage 2013: Tasting #4									
	Appear	Aroma	Acidity	Balance	Develop	Finish	Flavor	Overall	Final score
Vin. 1	1.78±0.44	1.89±1.05	1.00±0.00	1.44±0.53	1.67±0.50	1.11±0.78	1.56±0.78	1.33±0.71	**11.78**
Vin. 2	1.67±0.50	2.44±1.01	1.00±0.00	1.11±0.60	1.67±0.50	1.44±0.53	1.78±0.44	1.78±0.67	**12.89**
Vin. 3	1.44±0.53	2.67±0.50	0.89±0.33	1.33±0.71	1.67±0.50	1.44±0.53	2.00±0.50	1.67±0.50	**13.11**
Vin. 4	1.11±0.33	1.89±0.78	0.89±0.33	1.00±0.50	1.67±0.71	1.22±0.67	1.89±0.33	1.44±0.73	**11.11**
Vin. 5	1.56±0.53	2.11±1.05	0.89±0.33	1.44±0.53	1.56±0.73	1.33±0.50	2.00±0.87	1.44±0.88	**12.33**
Vin. 6	1.56±0.73	0.67±0.86	0.44±0.53	0.33±0.50	0.56±0.88	0.33±0.50	0.33±0.50	0.22±0.44	**4.44**
Vin. 7	1.33±0.50	2.22±0.83	0.78±0.44	1.22±0.83	1.56±0.73	1.33±0.71	1.67±0.50	1.44±0.73	**11.56**
Vin. 8	1.00±0.50	1.44±0.53	0.44±0.53	0.56±0.53	1.22±0.67	0.78±0.44	1.11±0.78	1.00±0.50	**7.56**
Vin. 9	1.00±0.50	0.67±0.71	0.56±0.53	0.56±0.73	0.67±0.50	0.56±0.73	0.22±0.44	0.33±0.50	**4.56**
Vin. 10	1.56±0.53	2.33±1.00	0.89±0.33	1.33±0.50	1.78±0.44	1.33±0.50	1.89±0.33	1.67±0.50	**12.78**
Vin. 11	1.78±0.44	2.11±0.60	0.89±0.33	1.56±0.53	1.78±0.67	1.44±0.73	1.78±0.44	1.56±0.53	**12.89**
Vin. 12	1.33±0.50	2.00±0.71	0.56±0.53	0.67±0.71	1.33±0.50	0.78±0.67	1.22±0.67	0.89±0.60	**8.78**
Vin. 13	1.00±0.50	1.89±0.78	0.89±0.33	1.00±0.50	1.22±0.44	0.89±0.33	1.33±0.50	1.33±0.50	**9.56**

(±) standard deviation of each individual tasting mean values (n=11).

Table A5: Wine sensory evaluation results of the four tasting sessions (vintage 2014). Final scores are the sum of scores of each attribute.

Vintage 2014: Tasting #1									
	Appear	Aroma	Acidity	Balance	Develop	Finish	Flavor	Overall	Final score
Vin. 1	2.00±0.00	2.56±1.33	0.89±0.33	1.33±0.87	1.67±0.50	1.44±0.53	1.78±0.44	1.89±0.60	**13.56**
Vin. 2	1.78±0.44	2.67±0.71	0.78±0.44	0.78±0.67	1.44±0.73	1.33±0.50	1.78±0.44	1.44±0.53	**12.00**
Vin. 3	1.44±0.53	3.11±0.60	0.78±0.44	1.44±0.53	1.67±0.50	1.56±0.53	1.89±0.33	1.67±0.50	**13.56**
Vin. 4	1.56±0.53	1.78±1.09	0.33±0.50	0.78±0.67	1.00±0.50	0.89±0.60	1.22±0.44	1.00±0.50	**8.56**
Vin. 5	1.11±0.60	1.67±0.87	0.44±0.53	0.67±0.50	1.00±0.00	0.56±0.53	1.11±0.60	0.78±0.44	**7.33**
Vin. 6	1.67±0.50	3.00±1.41	0.78±0.44	1.44±0.73	1.56±0.53	1.11±0.60	1.78±0.44	1.67±0.71	**13.00**
Vin. 7	1.89±0.33	2.44±1.36	0.89±0.33	1.11±0.60	1.56±0.53	1.33±0.50	1.67±0.87	1.67±0.71	**12.56**
Vin. 8	1.44±0.53	2.89±1.64	1.00±0.00	1.56±0.53	1.67±0.71	1.33±0.50	1.67±0.50	1.78±0.67	**13.33**
Vin. 9	1.67±0.50	2.22±0.93	0.89±0.33	1.11±0.60	1.56±0.73	1.11±0.60	1.67±0.71	1.78±0.83	**12.00**
Vin. 10	1.89±0.33	2.89±0.93	1.00±0.00	1.67±0.50	2.00±0.50	1.56±0.53	2.11±0.60	2.00±0.50	**15.11**
Vin. 11	1.78±0.44	2.89±0.60	0.89±0.33	1.56±0.53	2.00±0.71	1.56±0.53	2.00±0.71	1.89±0.60	**14.56**
Vin. 12	1.44±0.53	2.11±1.00	0.33±0.50	0.56±0.53	1.00±0.50	0.78±0.67	1.44±0.53	1.00±0.50	**8.67**
Vin. 13	1.78±0.44	2.67±1.00	0.89±0.33	1.11±0.60	1.89±0.60	1.33±0.60	1.89±0.60	1.67±0.71	**13.22**

Vintage 2014: Tasting #2									
	Appear	Aroma	Acidity	Balance	Develop	Finish	Flavor	Overall	Final score
Vin. 1	1.89±0.33	3.11±0.93	1.00±0.00	1.78±0.44	2.00±0.50	1.56±0.53	1.89±0,67	2.22±0.67	**15.44**
Vin. 2	1.67±0.50	2.56±0.88	0.89±0.33	1.33±0.50	1.89±0.60	1.22±0.44	1.59±0.71	1.67±0.87	**12.81**
Vin. 3	1.22±0.67	2.89±0.93	0.89±0.33	1.33±0.50	1.89±0.33	1.44±0.53	1.61±0.33	1.67±0.50	**12.94**
Vin. 4	1.44±0.53	1.67±1.12	0.67±0.50	0.89±0.33	1.22±0.44	0.89±0.33	1.13±0.44	1.00±0.00	**8.91**
Vin. 5	1.22±0.44	1.33±0.87	0.33±0.50	0.44±0.53	0.89±0.60	0.67±0.50	0.81±0.50	0.56±0.53	**6.26**
Vin. 6	1.78±0.44	3.00±1.22	0.89±0.33	1.22±0.44	1.56±0.53	1.22±0.44	1.61±0.50	1.44±0.53	**12.72**
Vin. 7	1.78±0.44	2.67±1.12	0.67±0.50	1.00±0.50	1.44±0.53	0.78±0.44	1.39±0.53	1.22±0.67	**10.94**
Vin. 8	1.22±0.67	2.56±0.88	0.89±0.33	1.33±0.50	1.67±0.71	1.44±0.53	1.52±0.60	1.67±0.71	**12.30**
Vin. 9	1.56±0.53	2.00±0.87	0.78±0.44	1.11±0.33	1.56±0.53	1.22±0.67	1.37±0.67	1.44±0.73	**11.04**
Vin. 10	1.78±0.44	2.89±0.93	0.89±0.33	1.44±0.53	1.89±0.33	1.56±0.53	1.74±0.50	1.67±0.70	**13.85**
Vin. 11	1.56±0.53	2.22±0.97	0.78±0.44	1.22±0.67	1.56±0.53	0.89±0.60	1.37±0.50	1.44±0.53	**11.04**
Vin. 12	1.11±0.60	1.67±0.87	0.22±0.44	0.33±0.50	0.89±0.60	0.56±0.53	0.80±0.50	0.78±0.44	**6.35**
Vin. 13	1.89±0.33	2.78±1.20	0.89±0.33	1.11±0.78	1.56±0.53	1.22±0.44	1.57±0.67	2.00±0.87	**13.02**

Vintage 2014: Tasting #3									
	Appear	Aroma	Acidity	Balance	Develop	Finish	Flavor	Overall	Final score
Vin. 1	2.00±0.00	2.80±0.40	1.00±0.00	1.40±0.49	2.20±0.75	1.40±0.40	2.20±0.49	2.40±0.49	**15.40**
Vin. 2	1.70±0.49	2.50±0.75	0.83±0.49	1.00±0.40	1.58±0.40	1.36±0.40	1.50±0.63	1.56±0.63	**12.03**
Vin. 3	1.20±0.75	2.60±1.20	1.00±0.00	1.60±0.49	2.40±0.49	1.60±0.74	2.20±0.49	2.20±0.75	**14.80**
Vin. 4	1.40±0.49	2.60±0.80	0.60±0.49	1.20±0.40	1.60±0.80	1.40±0.63	2.00±0.49	1.80±0.75	**12.60**
Vin. 5	1.40±0.49	1.60±0.49	0.60±0.49	0.80±0.40	1.20±0.40	1.00±0.49	1.60±0.63	1.00±0.63	**9.20**
Vin. 6	1.60±0.49	2.80±1.17	0.60±0.49	1.00±0.89	1.80±0.40	1.00±0.49	1.60±0.63	1.60±0.80	**12.00**
Vin. 7	1.60±0.49	2.60±1.36	0.80±0.40	1.20±0.75	1.60±0.80	1.40±0.40	1.80±0.49	1.80±0.98	**12.80**
Vin. 8	1.60±0.49	2.80±0.40	0.60±0.49	1.60±0.49	1.80±0.40	1.00±0.00	2.00±0.63	2.00±0.63	**13.40**
Vin. 9	1.80±0.40	1.60±.1.74	0.80±0.40	1.20±0.40	1.40±0.80	1.00±0.98	1.20±0.63	1.40±0.80	**10.40**
Vin. 10	2.00±0.00	3.40±0.80	1.00±0.00	1.40±0.49	2.20±0.75	1.40±0.00	2.00±0.49	2.20±0.75	**15.60**
Vin. 11	2.00±0.00	2.80±0.75	0.60±0.49	1.20±0.75	2.00±0.63	1.00±0.40	1.80±0.63	1.80±0.75	**13.20**
Vin. 12	1.20±0.74	1.80±0.75	0.20±0.40	0.80±0.40	1.00±0.00	0.80±0.40	1.20±0.40	1.00±0.00	**8.00**
Vin. 13	2.00±0.00	3.00±0.63	0.60±0.49	1.40±0.49	2.00±0.63	1.40±0.63	2.00±0.49	1.80±0.40	**14.20**

Vintage 2014: Tasting #4									
	Appear	Aroma	Acidity	Balance	Develop	Finish	Flavor	Overall	Final score
Vin. 1	1.80±0.45	2.40±1.14	0.80±0.45	1.40±0.55	2.00±0.71	1.80±0.45	2.20±0.45	2.00±0.71	**14.40**
Vin. 2	1.70±0.55	2.70±0.71	0.83±0.45	1.00±0.71	1.70±0.55	1.22±0.55	1.60±0.71	1.60±0.55	**12.35**
Vin. 3	1.60±0.55	3.60±0.89	0.80±0.45	1.60±0.55	2.00±1.00	1.40±0.54	2.40±089	2.20±0.84	**15.60**
Vin. 4	1.60±0.55	1.80±0.84	0.60±0.55	1.00±0.00	1.40±0.55	1.20±0.84	1.80±0.84	1.40±0.55	**10.80**
Vin. 5	1.80±0.45	1.40±1.14	0.60±0.55	1.00±0.71	1.40±0.55	1.20±0.45	1.40±0.55	1.00±0.71	**9.80**
Vin. 6	2.00±0.00	3.20±1.30	1.00±0.00	1.60±0.55	1.80±0.45	1.20±0.45	2.00±0.71	1.80±0.84	**14.60**
Vin. 7	1.80±0.45	2.80±0.45	1.00±0.00	1.60±0.55	2.00±0.00	1.60±0.55	2.00±0.00	2.40±0.55	**15.20**
Vin. 8	1.20±0.84	3.60±0.54	1.00±0.00	1.60±0.55	2.00±0.71	1.40±0.55	2.00±0.71	2.40±0.55	**15.20**
Vin. 9	1.40±0.55	1.20±1.10	0.80±0.45	1.20±0.84	1.40±0.55	1.00±0.00	1.20±0.45	1.20±0.45	**9.40**
Vin. 10	2.00±0.00	3.00±0.71	1.00±0.00	1.60±0.55	2.00±0.71	1.60±0.55	2.60±0.55	2.60±0.55	**16.40**
Vin. 11	1.80±0.45	3.00±0.71	0.60±0.55	1.00±0.71	1.60±1.14	1.40±0.84	1.80±0.84	1.80±0.84	**13.00**
Vin. 12	1.40±0.89	1.40±1.14	0.60±0.55	0.60±0.55	0.80±0.45	0.80±0.55	1.40±0.55	1.00±0.00	**8.00**
Vin. 13	1.80±0.45	3.00±0.71	0.80±0.45	1.80±0.45	2.20±1.10	1.20±0.55	2.60±0.55	1.80±0.84	**15.20**

(±) standard deviation of each individual tasting mean values (n=11).