A STUDY OF SEED DEVELOPMENT AND PHENOLIC COMPOUNDS IN SEEDS, SKINS AND WINES OF VITIS VINIFERA L. cv. SHIRAZ

Renata Ristic

The University of Adelaide
Faculty of Sciences
School of Agriculture and Wine
Discipline of Wine and Horticulture

A thesis submitted to the University of Adelaide in fulfilment of the requirement for a degree of Doctor of Philosophy
TABLE OF CONTENTS

CHAPTER 1 – GENERAL INTRODUCTION ............................................................... 1

1.1. SEED AND BERRY DEVELOPMENT .................................................................. 1
   1.1.1. Seed development ...................................................................................... 1
   1.1.2. Berry development .................................................................................. 3
   1.1.3. The influence of seeds on berry development and composition .............. 4

1.2. PHENOLIC COMPOUNDS IN THE GRAPE BERRY ............................................. 6
   1.2.1. Monomeric flavan-3-ols and tannins ....................................................... 7
   1.2.2. Anthocyanins ......................................................................................... 10
   1.2.3. Flavonols ............................................................................................... 11

1.3. INFLUENCE OF SUNLIGHT INTENSITY ON BERRY COMPOSITION .............. 12
   1.3.1. Influence of sunlight intensity on berry weight, total soluble solids, juice pH and titratable acidity (TA) .......................................................... 12
   1.3.2. Influence of sunlight intensity on phenolic composition of berries .......... 14

1.4. PHENOLIC COMPOUNDS IN WINES ............................................................ 16

1.5. PHENOLIC COMPOUNDS IN WINES AND WINE SENSORY PROPERTIES ..... 21

1.6. CANOPY MANIPULATION AND VINE CANOPY MICROCLIMATE .............. 24

1.7. AIMS OF THE STUDY ................................................................................. 26

CHAPTER 2 – EXPERIMENTAL DESIGN AND VINE CHARACTERISTICS 28

2.1. INTRODUCTION ......................................................................................... 28

2.2. MATERIALS AND METHODS ................................................................. 28
   2.2.1. Location and description of experimental site ......................................... 28
   2.2.2. Viticultural treatments and experimental design ...................................... 29
   2.2.3. Sampling procedure ............................................................................. 30
   2.2.4. General sample preparation .................................................................. 31
   2.2.5. Statistical methods .............................................................................. 31
   2.2.6. Vineyard parameters ........................................................................... 32

2.3. RESULTS ................................................................................................. 34
   2.3.1. Degree of sunlight intensity at the bunch zone ....................................... 34
   2.3.2. Canopy characteristics ....................................................................... 34
   2.3.3. Yield and vine balance ....................................................................... 36

2.4. DISCUSSION ......................................................................................... 37

2.5. CONCLUSION ....................................................................................... 40

CHAPTER 3 – DEVELOPMENTAL CHANGES IN MORPHOLOGICAL CHARACTERISTICS OF SEEDS AND THEIR RELATIONSHIPS TO BERRY DEVELOPMENT ........................ 41

3.1. INTRODUCTION ....................................................................................... 41

3.2. MATERIALS AND METHODS ................................................................. 41

3.3. RESULTS .............................................................................................. 42
   3.3.1. Developmental changes in the morphology of the grape seed ............... 42
CHAPTER 4 - DEVELOPMENTAL CHANGES IN PHENOLIC COMPOSITION OF SEEDS AND SKINS

4.1. INTRODUCTION ....................................................... 58
4.2. MATERIALS AND METHODS ........................................... 58
  4.2.1. Seed sample preparation ........................................ 58
  4.2.2. Berry sample preparation ...................................... 62
4.3. RESULTS ............................................................ 62
  4.3.1. Developmental changes in phenolic composition of seeds .... 62
  4.3.2. Developmental changes in the chemical composition of berries ... 71
  4.3.3. Relationships between developmental changes in seeds and berries ... 78
4.4. DISCUSSION .......................................................... 80
  4.4.1. Developmental changes in the phenolic composition of seeds and their relationships with seed and berry development .... 80
  4.4.2. Hypothesis about oxidation of seed tannins ...................... 83
  4.4.3. Influence of sunlight intensity at the bunch zone on the phenolic composition of seeds and berries .......................... 86
4.5. CONCLUSION ........................................................ 89

CHAPTER 5 - THE EFFECTS OF SUNLIGHT INTENSITY AT THE BUNCH ZONE ON PHENOLIC COMPOUNDS IN THE SKINS

5.1. INTRODUCTION .......................................................... 91
5.2. MATERIALS AND METHODS ............................................ 92
5.3. RESULTS ............................................................... 95
  5.3.1. Berry weight and concentration of total soluble solids (°Brix) .... 95
  5.3.2. The concentration and level of anthocyanins in the berry skin .......... 96
  5.3.3. The concentration, level and composition of monomeric flavan-3-ols and tannins in the skin ................................... 109
  5.3.4. The concentration and level of quercetin-3-glucoside and quercetin in the berry skin .......................................... 114
  5.3.5. Relationships between phenolic compounds in the berry .......... 117
5.4. DISCUSSION .......................................................... 118
  5.4.1. The concentration, level and composition of anthocyanins in the berry skin ......................................................... 119
  5.4.2. The concentration, level and composition of tannins in the berry skin ............................................................. 122
5.4.3. The concentration and level of quercetin and quercetin-3-glucoside in the berry skin .................................................. 124
5.4.4. Relationships between phenolic compounds in the berry skin and between phenolic compounds in berries and sunlight intensity at the bunch zone... 125
5.5. CONCLUSION ........................................................................... 127

CHAPTER 6 - PHENOLIC COMPOSITION AND SENSORY PROPERTIES OF WINES ................................................................. 129

6.1. INTRODUCTION ...................................................................... 129
6.2. MATERIAL AND METHODS ...................................................... 130
  6.2.1. Sampling procedure, general berry sample preparation and determination of berry colour, skin and seed tannins ............ 130
  6.2.2. Small-lot winemaking procedure and wine analysis .............. 130
  6.2.3. Wine chemical analysis .................................................... 131
  6.2.4. Sensory analysis ............................................................. 133
  6.2.5. Statistical methods for sensory analysis .............................. 136
6.3. RESULTS ............................................................................. 137
  6.3.1. Chemical composition of berries sampled for winemaking ..... 137
  6.3.2. Chemical composition of wines ......................................... 144
  6.3.3. Relationships between the phenolic composition of berries and the phenolic composition of wines .............................. 158
  6.3.4. Sensory evaluation of wines after 12 months of ageing .......... 161
6.4. DISCUSSION ........................................................................ 167
  6.4.1. Phenolic compounds in wines ........................................... 167
  6.4.2. Modified wine colour density and modified wine hue .......... 173
  6.4.3. Sensory properties of wines .............................................. 177
6.5. CONCLUSION ....................................................................... 182

CHAPTER 7 - GENERAL DISCUSSION AND DIRECTIONS FOR FUTURE RESEARCH ...................................................................... 184

7.1. THE BACKGROUND TO THE STUDY ........................................ 184
7.2. EXPERIMENTAL TREATMENTS AND VINE RESPONSE TO THE APPLIED CANOPY MANIPULATIONS ............................... 184
7.3. SEED DEVELOPMENT AND ITS RELATIONSHIPS WITH BERRY DEVELOPMENT .............................................. 185
7.4. DEVELOPMENTAL CHANGES IN PHENOLIC COMPOSITION OF SEEDS AND SKINS ..................................................... 187
7.5. INFLUENCE OF SUNLIGHT INTENSITY AT THE BUNCH ZONE ON THE PHENOLIC COMPOSITION OF SEEDS AND SKINS .......... 188
7.6. PHENOLIC COMPOSITION AND SENSORY PROPERTIES OF WINES ............................................................... 190

APPENDIX .................................................................................... 193

REFERENCES .............................................................................. 195
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABA</td>
<td>abscisic acid</td>
</tr>
<tr>
<td>°C</td>
<td>degrees Celsius</td>
</tr>
<tr>
<td>C</td>
<td>catechin</td>
</tr>
<tr>
<td>concentration</td>
<td>amount per gram berry weight</td>
</tr>
<tr>
<td>DAF</td>
<td>days after flowering</td>
</tr>
<tr>
<td>EC</td>
<td>epicatechin</td>
</tr>
<tr>
<td>EGC</td>
<td>epigallocatechin</td>
</tr>
<tr>
<td>ECG</td>
<td>epicatechin gallate</td>
</tr>
<tr>
<td>E-L</td>
<td>Eichhorn and Lorenz</td>
</tr>
<tr>
<td>EPR</td>
<td>electron paramagnetic resonance</td>
</tr>
<tr>
<td>FW</td>
<td>fruit weight (kg/vine)</td>
</tr>
<tr>
<td>FW/PW</td>
<td>ratio of fruit weight to pruning weight (kg/kg)</td>
</tr>
<tr>
<td>GA</td>
<td>gibberelic acid</td>
</tr>
<tr>
<td>LA</td>
<td>leaf area (m²)</td>
</tr>
<tr>
<td>LA/FW</td>
<td>ratio of leaf area/fruit weight (cm²/g)</td>
</tr>
<tr>
<td>level</td>
<td>amount per berry</td>
</tr>
<tr>
<td>LLA</td>
<td>lateral leaf area (m²)</td>
</tr>
<tr>
<td>M3G</td>
<td>malvidin-3-glucoside</td>
</tr>
<tr>
<td>mDP</td>
<td>mean degree of polymerisation</td>
</tr>
<tr>
<td>MLA</td>
<td>main leaf area (m²)</td>
</tr>
<tr>
<td>ns</td>
<td>not significant</td>
</tr>
<tr>
<td>PAR</td>
<td>photosynthetically active radiation</td>
</tr>
<tr>
<td>PCD</td>
<td>programmed cell death</td>
</tr>
<tr>
<td>PPFD</td>
<td>photosynthetic photon flux density</td>
</tr>
<tr>
<td>PPO</td>
<td>polyphenoloxidase</td>
</tr>
<tr>
<td>POD</td>
<td>peroxidase</td>
</tr>
<tr>
<td>PW</td>
<td>pruning weight (kg/vine)</td>
</tr>
<tr>
<td>r</td>
<td>coefficient of correlation</td>
</tr>
<tr>
<td>r²</td>
<td>coefficient of determination</td>
</tr>
<tr>
<td>ROS</td>
<td>reactive oxidative species</td>
</tr>
<tr>
<td>SA</td>
<td>canopy surface area</td>
</tr>
<tr>
<td>TA</td>
<td>titratable acidity (g/L as tartaric acid)</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>TSS</td>
<td>total soluble solids (°Brix)</td>
</tr>
<tr>
<td>v/v</td>
<td>volume to volume</td>
</tr>
<tr>
<td>Y</td>
<td>yield (kg/vine)</td>
</tr>
<tr>
<td>Y/PW</td>
<td>ratio of yield/pruning weight (kg/kg)</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

Figure 1.1. Line diagram of dorsal and ventral sides of a mature grape seed showing the beak, hilum, notch, fossettes, carina, raphe and chalaza. Modified from Buric (1985).......................... 2

Figure 1.2. A schematic diagram of the general flavonoid biosynthesis.......................... 6

Figure 1.3. Structure of flavan-3-ols: epicatechin, catechin, and epicatechin gallate........... 7

Figure 1.4. General structure of tannins formed from flavan-3-ols through C4-C8 or C4-C6 bond and a general structure of a trimer. Modified from Fuleki and Ricardo da Silva (1997).......................... 8

Figure 1.5. General structure of the anthocyanin aglycones........................................ 11

Figure 1.6. Structure of quercetin................................................................. 12

Figure 1.7. Effect of canopy density on berry anthocyanin production. Adapted from Iland (1989 b)................................................................. 14

Figure 1.8. Hypothetical mechanisms leading to tannin-tannin and tannin-anthocyanin additions with and without the presence of acetaldehyde. Modified from Cheynier et al. (2000).......................... 18

Figure 3.1. Linear diagram of developmental changes in the appearances of Shiraz grape seeds................................................................. 43

Figure 3.2. Changes in seed coat colour values during berry development and ripening under different light conditions. The berries of the following treatments: ST (1-10% sunlight at the bunch zone), MET (10-50% sunlight at the bunch zone) and HET (40-80% sunlight at the bunch zone) were sampled during (a) 1999/2000 and (b) 2000/2001 season. Each point represents the mean of eight replicates and error bars represent ± standard error of the mean.......................... 44

Figure 3.3. Changes in berry weight during berry development and ripening under different light conditions. The berries of the following treatments: ST (1-10% sunlight at the bunch zone), MET (10-50% sunlight at the bunch zone) and HET (40-80% sunlight at the bunch zone) were sampled during (a) 1999/2000 and (b) 2000/2001 season. Each point represents the mean of eight replicates and error bars represent ± standard error of the mean.......................... 46

Figure 3.4. Changes in weight of fresh seeds, dry seeds and water loss (difference between fresh and dry seed weight) during berry development and ripening under different light conditions. The berries of the following treatments: ST (1-10% sunlight at the bunch zone), MET (10-50% sunlight at the bunch zone) and HET (40-80% sunlight at the bunch zone) were sampled during (a) 1999/2000 and (b) 2000/2001 season. Each point represents the mean of eight replicates and error bars represent ± standard error of the mean.......................... 48
Figure 3.5.a. Relationship between berry weight and ♦ fresh and □ dry seed weight during berry development and ripening in (a) 1999/2000 and (b) 2000/2001 season. Polynomial curves are fitted for fresh seed weight (top line) and dry seed weight (bottom line) in each season using the data from all treatments. Significance of the coefficient determined by *** is significant at P<0.001. Note the different x-axis and y-axis scales. ................................................................. 50

Figure 3.5.b. Relationship between berry weight and fresh and dry seed weight during berry development and ripening in (a) 1999/2000 and (b) 2000/2001 season. Different periods of berry development and ripening are marked by colour of the seed coat as follows: ♦ (green) represents the period pre-veraison, ♠ (yellow) represents veraison and ◆ (brown) represents post-veraison. Polynomial curves are fitted for fresh and dry seed weight during each period in both seasons using the data from all treatments. ................................................................. 51

Figure 3.6. Notional diagram of the phases in the course of seed growth showing developmental changes in seed coat colour, formation of the seed features and changes in fresh seed weight, dry seed weight and water loss during berry development and ripening for the following treatments: ♦ ST (1-10% sunlight at the bunch zone), ♠ MET (10-50% sunlight at the bunch zone) and Δ HET (40-80% sunlight at the bunch zone). Each point represents the mean of eight replicates and error bars represent ± standard error of the mean. Curves are fitted for fresh weight, dry weight and water loss in (--) 1999/2000 and (---) 2000/2001 season ................................................................. 54

Figure 4.1. Developmental changes in the concentration and level of seed tannins during berry development and ripening under different light conditions. The berries of the following treatments: ♦ ST (1-10% sunlight at the bunch zone), ♠ MET (10-50% sunlight at the bunch zone) and Δ HET (40-80% sunlight at the bunch zone) were sampled during (a) 1999/2000 and (b) 2000/2001 season. Each point represents the mean of eight replicates and error bars represent ± standard error of the mean ................................................................. 63

Figure 4.2. Developmental changes in the concentration and level of extension subunits in seeds during berry development and ripening under different light conditions. The berries of the following treatments: ♦ ST (1-10% sunlight at the bunch zone), ♠ MET (10-50% sunlight at the bunch zone) and Δ HET (40-80% sunlight at the bunch zone) were sampled during (a) 1999/2000 and (b) 2000/2001 season. Each point represents the mean of eight replicates and error bars represent ± standard error of the mean ................................................................. 65

Figure 4.3. Developmental changes in the composition of extension subunits in seeds during berry development and ripening under different light conditions. The berries were sampled during (a) 1999/2000 and (b) 2000/2001 season. Each point represents the mean of 24 replicates and error bars represent ± standard error of the mean ................................................................. 65

Figure 4.4. Developmental changes in the concentration and level of terminal subunits in seeds during berry development and ripening under different light conditions. The berries of the following treatments: ♦ ST (1-10% sunlight at the bunch zone), ♠ MET (10-50% sunlight at the bunch zone) and Δ HET (40-80% sunlight at the bunch zone) were sampled during (a) 1999/2000 and (b) 2000/2001 season. Each point represents the mean of eight replicates and error bars represent ± standard error of the mean ................................................................. 66
Figure 4.5. Developmental changes in the composition of terminal subunits in seeds during berry development and ripening under different light conditions. The berries were sampled during (a) 1999/2000 and (b) 2000/2001 season. Each point represents the mean of 24 replicates and error bars represent ± standard error of the mean. ................................................................. 67

Figure 4.6. Developmental changes in the concentration and level of flavan-3-ol monomers in seeds during berry development and ripening under different light conditions. The berries of the following treatments: • ST (1-10% sunlight at the bunch zone), = MET (10-50% sunlight at the bunch zone) and Δ HET (40-80% sunlight at the bunch zone) were sampled during (a) 1999/2000 and (b) 2000/2001 season. Each point represents the mean of eight replicates and error bars represent ± standard error of the mean. ................................................................. 68

Figure 4.7. Developmental changes in the composition of flavan-3-ol monomers in seeds during berry development and ripening under different light conditions. The berries were sampled during (a) 1999/2000 and (b) 2000/2001 season. Each point represents the mean of 24 replicates and error bars represent ± standard error of the mean. ................................................................. 69

Figure 4.8. Developmental changes in polymer size of seed tannins during berry development and ripening under different light conditions. The berries of the following treatments: • ST (1-10% sunlight at the bunch zone), = MET (10-50% sunlight at the bunch zone) and Δ HET (40-80% sunlight at the bunch zone) were sampled during (a) 1999/2000 and (b) 2000/2001 season. Each point represents the mean of eight replicates and error bars represent ± standard error of the mean. ................................................................. 70

Figure 4.9. Developmental changes in juice total soluble solids (°Brix) during berry development and ripening under different light conditions. The berries of the following treatments: • ST (1-10% sunlight at the bunch zone), = MET (10-50% sunlight at the bunch zone) and Δ HET (40-80% sunlight at the bunch zone) were sampled during (a) 1999/2000 and (b) 2000/2001 season. Each point represents the mean of eight replicates and error bars represent ± standard error of the mean. ................................................................. 71

Figure 4.10.a. Developmental changes in concentration and level of total anthocyanins during berry ripening under different light conditions. The berries of the following treatments: • ST (1-10% sunlight at the bunch zone), = MET (10-50% sunlight at the bunch zone) and Δ HET (40-80% sunlight at the bunch zone) were sampled during (a) 1999/2000 and (b) 2000/2001 season. Each point represents the mean of eight replicates and error bars represent ± standard error of the mean. ................................................................. 73

Figure 4.10.b. Developmental changes in concentration and level of total anthocyanins of berries ripened under different light conditions in relation to changes in total soluble solids (°Brix). The berries of the following treatments: • ST (1-10% sunlight at the bunch zone), = MET (10-50% sunlight at the bunch zone) and Δ HET (40-80% sunlight at the bunch zone) were sampled during (a) 1999/2000 and (b) 2000/2001 season. Each point represents the mean of eight replicates and error bars represent ± standard error of the mean. Polynomial curves are fitted for each treatment. ................................................................. 74
Figure 4.11.a. Developmental changes in concentration and level of skin total phenolics during berry ripening under different light conditions. The berries of the following treatments: ⋄ ST (1-10% sunlight at the bunch zone), ■ MET (10-50% sunlight at the bunch zone) and Δ HET (40-80% sunlight at the bunch zone) were sampled during (a) 1999/2000 and (b) 2000/2001 season. Each point represents the mean of eight replicates and error bars represent ± standard error of the mean. 

Figure 4.11.b. Developmental changes in concentration and level of skin total phenolics of berries developed and ripened under different light conditions in relation to changes in total soluble solids (°Brix). The berries of the following treatments: ⋄ ST (1-10% sunlight at the bunch zone), ■ MET (10-50% sunlight at the bunch zone) and Δ HET (40-80% sunlight at the bunch zone) were sampled during (a) 1999/2000 and (b) 2000/2001 season. Each point represents the mean of eight replicates and error bars represent ± standard error of the mean. Polynomial curves are fitted for each treatment.

Figure 4.12. The relationships between the seed coat colour value and the level of seed tannins, the level of total anthocyanins, the level of skin total phenolics during the period from the beginning of veraison until harvest using data from all treatments. Significance of the coefficient of determination indicated by *** is significant at P<0.001. Note the different y-axis scales.

Figure 4.13. Notional diagram of seed and berry development showing how the sequences of seed development coincide with the sequences of berry development: i) seed formation and berry formation were completed by the beginning of veraison when the maximum level of seed tannins and maximal fresh seed weight were observed (60 DAF) ii) the level of monomers was at a maximum and the rapid oxidation of seed tannins commenced at veraison (70 DAF) iii) formation of all seed features (the beak, chalaza and raphé) was completed, dry seed weight was at a maximum, rapid oxidation of seed tannins finished and berries reached their maximum weight (92 DAF).

Figure 4.14. Developmental changes in the concentration and level of seed tannins in berries developed and ripened under different light conditions in relation to changes in total soluble solids (°Brix). The berries of the following treatments: ⋄ ST (1-10% sunlight at the bunch zone), ■ MET (10-50% sunlight at the bunch zone) and Δ HET (40-80% sunlight at the bunch zone) were sampled during (a) 1999/2000 and (b) 2000/2001 season. Each point represents the mean of eight replicates and error bars represent ± standard error of the mean. Polynomial curves are fitted for each treatment.

Figure 5.1. The concentration and level of (a) total anthocyanins, (b) anthocyanins in the mono-glucoside form, (c) anthocyanins in the acetyl-glucoside form and (d) anthocyanins in the coumaroyl-glucoside form in the skins of berries developed and ripened under different light conditions. The berries of the following treatments: # BT (0 % sunlight at the bunch zone), ⋄ ST (1-10% sunlight at the bunch zone), ■ MET (10-50% sunlight at the bunch zone) and Δ HET (40-80% sunlight at the bunch zone) were sampled in the 2000 and 2001 season. Error bars represent ± standard error of the mean (n=8). Different letters above error bars indicate significant difference (P<0.05) between means within one season. Note the different y-axis scales.
Figure 5.2. The concentration of individual anthocyanins in the mono-glucoside form: (a) delphinidin mono-glucoside, (b) cyanidin mono-glucoside, (c) petunidin mono-glucoside, (d) peonidin mono-glucoside and (e) malvidin mono-glucoside in the coumaroyl-glucoside form in the skins of berries developed and ripened under different light conditions. The berries of the following treatments: # BT (0 % sunlight at the bunch zone), ■ ST (1-10% sunlight at the bunch zone), ◆ MET (10-50% sunlight at the bunch zone) and □ HET (40-80% sunlight at the bunch zone) were sampled in the 2000 and 2001 season. Error bars represent ± standard error of the mean (n=8). Different letters above error bars indicate significant difference (P<0.05) between means within one season. Note the different y-axis scales. ................................................................. 100

Figure 5.3. The concentration of individual anthocyanins in the acetyl-glucoside form: (a) delphinidin acetyl-glucoside, (b) cyanidin acetyl-glucoside, (c) petunidin acetyl-glucoside, (d) peonidin acetyl-glucoside and (e) malvidin acetyl-glucoside in the skin of berries developed and ripened under different light conditions. The berries of the following treatments: # BT (0 % sunlight at the bunch zone), ■ ST (1-10% sunlight at the bunch zone), ◆ MET (10-50% sunlight at the bunch zone) and □ HET (40-80% sunlight at the bunch zone) were sampled in the 2000 and 2001 season. Error bars represent ± standard error of the mean (n=8). Different letters above error bars indicate significant difference (P<0.05) between means within one season. Note the different y-axis scales. ................................................................. 102

Figure 5.4. The concentration of individual anthocyanins in the coumaroyl-glucoside form: (a) delphinidin coumaroyl-glucoside, (b) cyanidin coumaroyl-glucoside, (c) petunidin coumaroyl-glucoside, (d) peonidin coumaroyl-glucoside and (e) malvidin coumaroyl-glucoside in the skin of berries developed and ripened under different light conditions. The berries of the following treatments: # BT (0 % sunlight at the bunch zone), ■ ST (1-10% sunlight at the bunch zone), ◆ MET (10-50% sunlight at the bunch zone) and □ HET (40-80% sunlight at the bunch zone) were sampled in the 2000 and 2001 season. Error bars represent ± standard error of the mean (n=8). Different letters above error bars indicate significant difference (P<0.05) between means within one season. Note the different y-axis scales. ................................................................. 104

Figure 5.5.a. The concentration of total anthocyanins, anthocyanins in the mono-glucoside form, anthocyanins in the acetyl-glucoside form and anthocyanins in the coumaroyl-glucoside form in relation to the sunlight interception at the bunch zone. The berries of the following treatments: ○ BT (0 % sunlight at the bunch zone), ♦ ST (1-10% sunlight at the bunch zone), ◆ MET (10-50% sunlight at the bunch zone) and □ HET (40-80% sunlight at the bunch zone) were sampled in (a) 2000 and (b) 2001 season Significance of the coefficient of determination is determined by: *, **, *** significant at P<0.05, P<0.01 and P<0.001.......... 107

Figure 5.5.b. The level of total anthocyanins, anthocyanins in the mono-glucoside form, anthocyanins in the acetyl-glucoside form and anthocyanins in the coumaroyl-glucoside form in relation to the sunlight interception at the bunch zone. The berries of the following treatments: ○ BT (0 % sunlight at the bunch zone), ♦ ST (1-10% sunlight at the bunch zone), ◆ MET (10-50% sunlight at the bunch zone) and □ HET (40-80% sunlight at the bunch zone) were sampled in (a) 2000 and (b) 2001 season Significance of the coefficient of determination is determined by: *, **, *** significant at P<0.05, P<0.01 and P<0.001. .......... 108
Figure 5.6. The concentration and level of (a) total tannins, (b) extension subunits, (c) terminal subunits and (d) monomer flavan-3-ols in the skin of berries developed and ripened under different light conditions. The berries of the following treatments: # BT (0 % sunlight at the bunch zone), ■ ST (1-10% sunlight at the bunch zone), □ MET (10-50% sunlight at the bunch zone) and △ HET (40-80% sunlight at the bunch zone) were sampled in the 2000 and 2001 season. Error bars represent ± standard error of the mean (n=8). Different letters above error bars indicate significant difference (P<0.05) between means within one season. Note the different y-axis scales. ........................................................................110

Figure 5.7. The concentration and level of total skin tannins in relation to sunlight interception at the bunch zone. The berries of the following treatments: ○ BT (0 % sunlight at the bunch zone), ♦ ST (1-10% sunlight at the bunch zone), ■ MET (10-50% sunlight at the bunch zone) and △ HET (40-80% sunlight at the bunch zone) were sampled in (a) 2000 and (b) 2001 season. Significance of the coefficient of determination is determined by: *, **, *** significant at P<0.05, P<0.01 and P<0.001............................................................................................................113

Figure 5.8. The concentration and level of quercetin-3-glucoside and quercetin in the skin of berries developed and ripened under different light conditions. The berries of the following treatments: # BT (0 % sunlight at the bunch zone), ■ ST (1-10% sunlight at the bunch zone), □ MET (10-50% sunlight at the bunch zone) and △ HET (40-80% sunlight at the bunch zone) were sampled in (a) 2000 and (b) 2001 season. Error bars represent ± standard error of the mean (n=8). Different letters above error bars indicate significant difference (P<0.05) between means within one season. Note the different y-axis scales. ........................................................................115

Figure 5.9. The concentration and level of quercetin-3-glucoside and quercetin in the berry skin in relation to sunlight interception at the bunch zone. The berries of the following treatments: ○ BT (0 % sunlight at the bunch zone), ♦ ST (1-10% sunlight at the bunch zone), ■ MET (10-50% sunlight at the bunch zone) and △ HET (40-80% sunlight at the bunch zone) were sampled in (a) 2000 and (b) 2001 season. Significance of the coefficient of determination is determined by: *, **, *** significant at P<0.05, P<0.01 and P<0.001............................................................................................................116

Figure 5.10. The relationships between the concentration of skin tannins and (a) the concentration of total anthocyanins and (b) the concentration of flavonoids in ♦ 2000 and ♦ 2001 season. Solid lines represent the linear relationship between variables in each season. Significance of the correlation coefficient is determined by **, *** significant at P<0.01 and P<0.001..............................117

Figure 5.11. The level of skin tannins, anthocyanins and seed tannins in the berry in relation to sunlight interception at the bunch zone (PPFD). The berries of the following treatments: ♦ ST (1-10% sunlight at the bunch zone), ■ MET (10-50% sunlight at the bunch zone) and △ HET (40-80% sunlight at the bunch zone) were sampled in (a) 2000 and (b) 2001 season. Polynomial curves were fitted for each group of phenolic compounds.................................................................126

Figure 6.1. The concentration of (a) total anthocyanins, (b) anthocyanins in the monoglucoside form, (c) anthocyanins in the acetyl-glucoside form and (d) anthocyanins in the coumaroyl-glucoside form in the berry skin at harvest. The berries of the following treatments: ST (1-10% sunlight at the bunch zone), MET (10-50% sunlight at the bunch zone), HET (40-80% sunlight at the bunch zone) and RT were sampled for winemaking in the 2000 and 2001 season. Error bars represent ± standard error of the mean (n=3). Different letters above error
bars indicate significant difference \((P<0.05)\) between means within one season. Note the different y-axis scales. ................................................................. 139

Figure 6.2. The concentration of (a) total tannins, (b) extension subunits, (c) terminal subunits and (d) monomeric flavan-3-ols in the berry skin at harvest. The berries of the following treatments: ST (1-10% sunlight at the bunch zone), MET (10-50% sunlight at the bunch zone), HET (40-80% sunlight at the bunch zone) and RT were sampled for winemaking in the 2000 and 2001 season. Error bars represent ± standard error of the mean \((n=3)\). Different letters above error bars indicate significant difference \((P<0.05)\) between means within one season. Note the different y-axis scales. ................................................................. 140

Figure 6.3. The concentration of (a) quercetin-3-glucoside and (b) quercetin in the berry skin at harvest. The berries of the following treatments: ST (1-10% sunlight at the bunch zone), MET (10-50% sunlight at the bunch zone), HET (40-80% sunlight at the bunch zone) and RT were sampled for winemaking in the 2000 and 2001 season. Error bars represent ± standard error of the mean \((n=3)\). Different letters above error bars indicate significant difference \((P<0.05)\) between means within one season. Note the different y-axis scales. ................................................................. 141

Figure 6.4. The concentration of (a) total tannins, (b) extension subunits, (c) terminal subunits and (d) monomeric flavan-3-ol in seeds of berries at harvest. The berries of the following treatments: ST (1-10% sunlight at the bunch zone), MET (10-50% sunlight at the bunch zone), HET (40-80% sunlight at the bunch zone) and RT were sampled for winemaking in the 2000 and 2001 season. Error bars represent ± standard error of the mean \((n=3)\). Different letters above error bars indicate significant difference \((P<0.05)\) between means within one season. Note the different y-axis scales. ................................................................. 143

Figure 6.5. The concentration of (a) total anthocyanins, (b) anthocyanins in the mono-glucoside form, (c) anthocyanins in the acetyl-glucoside form and (d) anthocyanins in the coumaroyl-glucoside form in wines after fermentation and after 12 months of ageing. Wines were made from berries of the following treatments: ST (1-10% sunlight at the bunch zone), MET (10-50% sunlight at the bunch zone), HET (40-80% sunlight at the bunch zone) and RT in the 2000 and 2001 season. Error bars represent ± standard error of the mean \((n=3)\). Different letters above error bars indicate significant difference \((P<0.05)\) between means within one season. Note the different y-axis scales. ................................................................. 146

Figure 6.6. The concentration of total flavan-3-ol monomers (catechin, epicatechin and epicatechin gallate) in wines after fermentation and after 12 months of ageing. Wines were made from berries of the following treatments: ST (1-10% sunlight at the bunch zone), MET (10-50% sunlight at the bunch zone), HET (40-80% sunlight at the bunch zone) and RT in the 2000 and 2001 season. Error bars represent ± standard error of the mean \((n=3)\). Different letters above error bars indicate significant difference \((P<0.05)\) between means within one season. Note the different y-axis scales. ................................................................. 148

Figure 6.7. The concentration of quercetin-3-glucoside and quercetin in wines after fermentation and after 12 months of ageing. Wines were made from berries of the following treatments: ST (1-10% sunlight at the bunch zone), MET (10-50% sunlight at the bunch zone), HET (40-80% sunlight at the bunch zone) and RT in the 2000 and 2001 season. Error bars represent ± standard error of the mean \((n=3)\). Different letters above error bars indicate significant difference \((P<0.05)\) between means within one season. Note the different y-axis scales. ................................................................. 149
Figure 6.8. The concentration of polymeric pigments in wines after fermentation and after 12 months of ageing. Wines were made from berries of the following treatments: ST (1-10% sunlight at the bunch zone), MET (10-50% sunlight at the bunch zone), HET (40-80% sunlight at the bunch zone) and RT in the 2000 and 2001 season. Error bars represent ± standard error of the mean (n=3). Different letters above error bars indicate significant difference (P<0.05) between means within one season. ........................................ 150

Figure 6.9. The concentration of tannins in wines after fermentation and after 12 months of ageing. Wines were made from berries of the following treatments: ST (1-10% sunlight at the bunch zone), MET (10-50% sunlight at the bunch zone), HET (40-80% sunlight at the bunch zone) and RT in the 2000 and 2001 season. Error bars represent ± standard error of the mean (n=3). Different letters above error bars indicate significant difference (P<0.05) between means within one season. 150

Figure 6.10. The relationship between wine colour density at wine pH, and wine colour density at adjusted wine pH to 3.60 and modified wine colour density at adjusted wine pH to 3.60 and an addition of acetaldehyde, measured and calculated for wines after fermentation and after 12 months of ageing. ......................... 152

Figure 6.11. Mean ratings for mouth-feel attributes in wines from the 2000 vintage. Wines were made from berries of the following treatments: ST (1-10% sunlight at the bunch zone), MET (10-50% sunlight at the bunch zone), HET (40-80% sunlight at the bunch zone) and RT. Each value is the mean score from two fermentation replicate wines that were presented to 14 judges in three replicate sessions. LSD is the least significant difference. ***,*** indicate significance at P<0.05, P<0.01, P<0.001 respectively. ................................................................. 162

Figure 6.12. Mean ratings for mouth-feel attributes in wines from the 2001 vintage. Wines were made from berries of the following treatments: ST (1-10% sunlight at the bunch zone), MET (10-50% sunlight at the bunch zone), HET (40-80% sunlight at the bunch zone) and RT. Each value is the mean score from three fermentation replicate wines that were presented to 13 judges in three replicate sessions. LSD is the least significant difference. ***,*** indicate significance at P<0.05, P<0.01, P<0.001 respectively......................................................... 163

Figure 6.13. Mean ratings for aroma attributes in wines from the 2001 vintage. Wines were made from berries of the following treatments: ST (1-10% sunlight at the bunch zone), MET (10-50% sunlight at the bunch zone), HET (40-80% sunlight at the bunch zone) and RT. Each value is the mean score from three fermentation replicate wines that were presented to 13 judges in three replicate sessions. LSD is the least significant difference ***,*** indicate significance at P<0.05, P<0.01, P<0.001 respectively......................................................... 164

Figure 6.14. The principal component analysis for the mean sensory profile of wines from the (00) 2000 and (01) 2001 vintage. Wines were made from berries of the following treatments: ST (1-10% sunlight at the bunch zone), MET (10-50% sunlight at the bunch zone), HET (40-80% sunlight at the bunch zone) and RT, in the 2000 and 2001 season. Each value for wines from the 2000 vintage is the mean score from two fermentation replicate wines that were presented to 14 judges in three replicate sessions. Each value for wines from the 2001 vintage is the mean score from three fermentation replicate wines that were presented to 13 judges in three replicate sessions......................................................... 165
Figure 6.15. The assessment of red wine colour and purple hue in wines from the 2001 vintage. Wines were made from berries of the following treatments: ST (1-10% sunlight at the bunch zone), MET (10-50% sunlight at the bunch zone), HET (40-80% sunlight at the bunch zone) and RT in the 2000 and 2001 season. Error bars represent ± standard error of the mean (n=3). Different letters above error bars indicate significant difference (P<0.05) between means for red wine colour and purple hue separately. .......................................................... 167

Figure 6.16. The relationship between UV/visible spectroscopy method of Somers and Evans and the HPLC method for determining the concentration of (a) total anthocyanins in wines, (b) polymeric pigments in wines and total phenolics/total tannin polymers (c) in wines after fermentation and (d) after 12 months of ageing, using the data from all the treatments. Significance of the correlation coefficient determined by *** is significant at P<0.001......................... 177
LIST OF TABLES

Table 2.1. Parameters of sunlight intensity at the bunch zone, canopy growth and canopy size. Measures were taken for the ST, MET and HET treatment in the 1999/2000 and 2000/2001 season. Each value represents the mean of 8 replicates. Means in columns followed by different letters are significantly different within one season. ................................................................. 35

Table 2.2. The average number of bunches per vine and per shoot, average bunch weight and yield per vine. Bunches of the ST, MET and HET treatment were sampled in the 1999/2000 and 2000/2001 season. Each value represents the mean of 8 replicates. Means in columns followed by different letters are significantly different within one season. ........................................................................... 37

Table 3.1. Berry weight, fresh and dry seed weight and seed dimensions of seeds from green and red coloured berries at veraison (64 DAF). Each value represents the mean of 30 berries of 24 replicates. Means in columns (± standard error of the mean) followed by different letters are significantly different. ........................................................................... 52

Table 5.1. The mean berry weight and the concentration of total soluble solids. The berries of the ST, MET, HET and BT treatment were sampled in the 2000 and 2001 season. Each value represents the mean of 8 replicates. Means in columns (± standard error of the mean) followed by different letters are significantly different within one season. .......... 96

Table 5.2. Proportions of anthocyanins in the mono-glucoside form, acetyl-glucoside form and coumaroyl-glucoside form in the skins of berries developed and ripened under different light conditions. The berries of the ST, MET, HET and BT treatment were sampled in the 2000 and 2001 season. Each value represents the mean of 8 replicates. Means in columns followed by different letters are significantly different within one season. ................................................................. 98

Table 5.3. Proportions of individual anthocyanins in the mono-glucoside form in the skins of berries developed and ripened under different light conditions. The berries of the ST, MET, HET and BT treatment were sampled in the 2000 and 2001 season. Each value represents the mean of 8 replicates. Means in columns followed by different letters are significantly different within one season. ............ 101

Table 5.4. Proportions of individual anthocyanins in the acetyl-glucoside form in the skins of berries developed and ripened under different light conditions. The berries of the ST, MET, HET and BT treatment were sampled in the 2000 and 2001 season. Each value represents the mean of 8 replicates. Means in columns followed by different letters are significantly different within one season. ............. 103

Table 5.5. Proportions of individual anthocyanins in the coumaroyl-glucoside form in the skins of berries developed and ripened under different light conditions. The berries of the ST, MET, HET and BT treatment were sampled in the 2000 and 2001 season. Each value represents the mean of 8 replicates. Means in columns followed by different letters are significantly different within one season. ............. 105
Table 5.6. Proportions of catechin (C), epicatechin (EC), epigallocatechin (EGC) and epicatechin gallate (ECG) in the extension and terminal subunits and monomers in the skins of berries developed and ripened under different light conditions. The berries of the ST, MET, HET and BT treatment were sampled in the 2000 and 2001 season. Each value represents the mean of 8 replicates. Means in columns followed by different letters are significantly different within one season.

Table 6.1. Composition of the aroma reference standards ........................................... 134

Table 6.2. The finger touch standards .............................................................................. 134

Table 6.3. The aroma and mouth-feel sensory attributes used for wines made in the (1) 2001 and (2) 2002 vintage................................................................................................. 135

Table 6.4. Berry weight, seed weight, seed number, juice total soluble solids (°Brix), juice pH and the concentration of total anthocyanins and skin total phenolics of berries at harvest in the 2000 and 2001 season. Each value represents the mean of 3 replicates. Means in columns followed by different letters are significantly different within one season................................................................. 138

Table 6.5. Ratio between the concentrations of skin and seed tannins, and anthocyanins in berries at harvest. The berries of the ST, MET, HET and RT treatment were sampled for winemaking in the 2000 and 2001 season. Each value represents the mean of 3 replicates. Means in columns followed by different letters are significantly different within one season................................................................. 144

Table 6.6. Proportion of anthocyanins in the mono-glucoside form, acetyl-glucoside form and coumaroyl-glucoside form in wines from the 2000 and 2001 season after fermentation and after 12 months of ageing. Each value represents the mean of 3 fermentation replicates. Means in columns followed by different letters are significantly different within one season................................................................. 147

Table 6.7. Wine colour density at wine pH (1), wine colour density at adjusted wine pH to 3.60 (2) and wine colour density at adjusted wine pH to 3.60 and the addition of acetaldehyde (3) of wines after fermentation and after 12 months of ageing. Each value represents the mean of 3 fermentation replicate. Means in rows followed by different letters are significantly different........................................... 151

Table 6.8. Modified wine colour density, modified wine hue, concentration of pigmented polymers, total anthocyanins and total phenolics in wines after fermentation and after 12 months of ageing. Each value represents the mean of 3 fermentation replicates. Means in columns followed by different letters are significantly different within one season................................................................. 153

Table 6.9. The chemical composition of wines after 12 months of ageing. Wines were made from berries of the following treatments: ST, MET, HET and RT in the 2000 and 2001 season. Each value represents the mean of 3 replicates. Means in columns followed by different letters are significantly different within one season................................................................. 157

Table 6.10. The correlation coefficients (r) of the relationships between the concentration of anthocyanins in the berry skin, as determined by the HPLC method and the UV/visible spectroscopy method, and modified wine colour density, modified wine hue, concentration of total anthocyanins, phenolics, polymeric pigments
and polymeric tannins in wines after fermentation and after 12 months of ageing. .................................................. 158

Table 6.11. The correlation coefficients (r) of the relationships between the concentration of phenolics in the berry as determined by the HPLC method and UV/visible spectrophotometry and the wine colour density, wine hue, concentration of total anthocyanins, phenolics, polymeric pigments and polymeric tannins in wines after fermentation and after 12 months of ageing. .................................................. 159

Table 6.12. The correlation coefficients (r) of the relationships between modified wine colour density and the concentration of anthocyanins, phenolics, polymeric pigments and tannins in wines after fermentation and after 12 months of ageing, as determined by the HPLC method and the UV/visible spectroscopy method. .... 161

Table 6.13. The mean score (± standard error of the mean) on a 20-point scale of wines after 12 months of ageing from the 2000 and 2001 vintage. Each value represents the mean of 2 (2000 vintage) or 3 (2001) replicates. Means in columns followed by different letters are significantly different within the same season. ......................... 166
Summary

This study investigated seed development, phenolic composition of seeds and skins, and phenolic composition and sensory properties of wines made from berries of *Vitis Vinifera* L. cv. Shiraz that were developed and ripened under different levels of sunlight intensity at the bunch zone. The study also examined the relationships between seed and berry development, and the links between berry composition, wine composition and wine sensory properties.

The experiment consisted of three main treatments comprising different levels of sunlight intensity at the bunch zone which were obtained by canopy manipulations. The treatments were as follows: i) shaded treatment (ST) (whole vine shading by wrapping vine canopies in bird nets to constrict the canopy), ii) moderately exposed treatment (MET) (no canopy manipulation) and iii) highly exposed treatment (HET) (vine canopies were divided and shoots were trained upwards and downwards). The additional treatments included i) box treatment (BT) in the 2000/2001 season (bunches in the zone of highly exposed bunches were enclosed in specially constructed boxes to exclude sunlight) and ii) reference treatment (RT) (vines in an adjacent row mananged to produce high quality fruit). The experiment was conducted for two growing seasons, 1999/2000 and 2000/2001.

The degree of sunlight intensity at the bunch zone differed highly significantly (*P*<0.001) between treatments indicating that the applied treatments were successful in creating variation in the amount of sunlight intercepted by the grape berries. Measures of light intensity at the bunch zone showed that the fruit of ST received less than 5% of ambient (<100 PPFD), MET 10-40% of ambient (300-700 PPFD), HET 40-80% of ambient (800-1500 PPFD) and BT 0%.

The patterns of developmental changes in fresh seed weight and dry seed weight followed biphasic curves. Grape seed growth was able to be divided into three phases: i) a phase of seed growth, ii) an intermediate phase and iii) a phase of seed drying and maturation. The definition of these phases was based on developmental changes in fresh seed weight, dry seed weight, water content, seed width and length and physical appearance of seeds that included formation of seed features and seed coat colour.
This part of the study also provided further evidence that seed and berry development occurred simultaneously and that seed development may affect berry development. It was observed that sequences of seed development coincided with sequences of berry development. Seeds reached maximum fresh seed weight and full size at the beginning of berry colouring (veraison). Maximum dry seed weight and complete formation of seed features coincided with maximum berry weight.

A seed colour chart was developed to describe and to provide a qualitative measure of the changes in colour of the seed coat. Changes of the seed coat colour during seed and berry development were highly correlated to both changes in the phenolic composition of seeds and changes in the phenolic composition of skins. The strong inverse correlation between seed coat colour values and the concentrations of extracted seed monomers and tannins suggested that during seed development seed coat and phenolic compounds underwent many chemical changes. These changes may be characterised as the natural sequences of seed maturation which lead to increased impermeability of the seed coat and seed dormancy. A hypothesis was developed about a possible mechanism of oxidation of phenolic compounds and their role in seed dormancy. Developmental changes of the seed coat colour were also highly correlated with developmental changes in the accumulation of total anthocyanins and skin total phenolics, indicating that the external appearance and colour of the seed coat may be used as an additional indicator of seed maturity and overall berry ripeness.

Different levels of sunlight intensity at the bunch zone had only a small effect on berry weight, seed weight and number of seeds per berry. Reduced sunlight intensity at the bunch zone (ST) delayed ripening and decreased the accumulation of total anthocyanins and skin total phenolics. During berry development, at the same stage of berry maturity (°Brix), the concentration of anthocyanins and skin total phenolics were similar in berries developed and ripened under moderate sunlight intensity (10-40% of ambient) (MET) to those berries developed and ripened under high light intensity (40-80% of ambient).

In the two years of the experiment the modification of the degree of light interception at the bunch zone had little effect on the level and concentration of seed tannins.
The concentration and composition of phenolic compounds in the skins (anthocyanins, skin tannins and flavonols) of berries of different treatments was compared at the stage of maximal concentration of total anthocyanins. The additional treatment of complete bunch shading (BT) was also included in this experiment in the second season. Excluded (BT) or low sunlight intensity (<100 PPFD) (ST) at the bunch zone affected the accumulation of phenolic compounds in the berry skins and the following effects were observed: i) reduced accumulation of total anthocyanins, with increased proportion of anthocyanins in the coumaroyl-glucoside form relative to those in the mono-glucoside form, ii) reduced accumulation of skin tannins, and iii) reduced accumulation of flavonols (quercetin and quercetin-3-glucoside).

The accumulation of total anthocyanins did not increase with increasing sunlight intensity above 50% of ambient (800-900 PPFD) which could be attributed to the high temperature effect of fully exposed berries. Most sensitive to changes in light and temperature conditions were anthocyanins in the mono-glucoside form, followed by anthocyanins in the coumaroyl-glucoside form and acetyl-glucoside form. The accumulation of skin tannins was enhanced with increasing sunlight intensity at the bunch zone until approximately 80-90% of ambient (1300 PPFD). The accumulation of quercetin and quercetin-3-glucoside was higher with increased sunlight intensity at the bunch zone indicating that the production of both compounds is highly dependent on light intensity. The results from this study indicated that the concentrations of anthocyanins, skin tannins and flavonols in the berry skins depend on complex interactions between light and temperature effects on berries. Strong relationships among the concentration of anthocyanins, tannins and quercetin and quercetin-3-glucoside in the berry skins indicated that accumulation of these compounds paralleled each other, but their coordination has yet to be established.

Wines were made from the three main treatments (ST, MET, HET) and the additional RT treatment, when berries reached approximately 23-25 °Brix. The contribution of seed components to wine made from berries of different treatments was similar within the same season. Berries from the moderately and highly exposed canopy conditions collected for winemaking had similar or higher concentration of anthocyanins and higher concentration of skin tannins. Berries of RT had higher concentration of anthocyanins and skin tannins.
Modified wine colour density, the concentration of total anthocyanins (only in the second season), polymeric pigments and tannin polymeric fraction, and total phenolics were significantly \((P<0.001)\) lower in wines of ST than in wines of MET and HET. Wines from berries grown under moderate sunlight intensity had similar concentration of total anthocyanins and total phenolics, but higher concentration of polymeric pigments and tannin polymers than wines from berries grown under high sunlight intensity. Wines of RT were significantly higher \((P<0.001)\) in modified wine colour density, the concentration of total anthocyanins, polymeric pigments, tannins and total phenolic than all other treatments.

The astringency-related mouth-feel sensory attributes (coarseness, grainy, puckery, adhesiveness), as well as the intensity and persistence of fruit flavour were rated higher in RT, MET and HET wines than ST wines and these effects were more pronounced in the second season.

The series of investigations provided the following information:

i) The differences in the concentration of anthocyanins in the berry skins between treatments were reflected in concomitant differences in the concentration of total anthocyanins in wines. During fermentation and wine maturation degradation of anthocyanins acylated with acetic acid was greater than those acylated with \(p\)-coumaric acid. A lower decrease in the concentration of non-acylated anthocyanins could be due to hydrolysis of acylated anthocyanins that compensated for the loss of non-acylated anthocyanins.

ii) The concentration of pigmented polymers and tannins in wines was related to the concentration and composition of anthocyanins and also to the concentration of seed and skin tannins, and the ratios (balance) between anthocyanins and tannins. Higher wine quality may be related to viticultural conditions which result in higher concentrations of anthocyanins and skin tannins in berries, coupled with a lower ratio of seed tannins to anthocyanins and a higher ratio of skin tannins to anthocyanins.

iii) Modified wine colour density was related to the concentration of polymeric pigments and anthocyanins in wines. The differences in modified wine colour density between treatments were greater than the differences in the concentration of total anthocyanins in berries.
iv) The astringency related mouth-feel attributes were related to the concentration of tannins in wines.

v) In general wines with higher concentration of phenolic compounds (anthocyanins, pigmented and non-pigmented polymers) and higher modified wine colour density were rated higher for most of the mouth feel attributes as well as higher on a quality scale.

Wine composition was related to the level and balance between anthocyanins, seed and skin tannins in berries. Excessive canopy shade was detrimental to berry composition, wine composition and wine sensory properties. In warm to hot viticultural climates adoption of practices which lead to moderate degree of bunch exposure are recommended. Thus, an assessment of canopy microclimate, in conjunction with berry composition needs to be undertaken to predict wine quality.
STATEMENT

This thesis contains no material which has been accepted for an award of any degree or diploma in any University and to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference is made in the text.

I give my consent to this copy of my thesis, when deposited in the University library, being available for loan and photocopying.

Signed:...

Renata Ristic
ACKNOWLEDGEMENTS

My supervisors Dr. Patrick Ilard, Dr. Leigh Francis and Dr. Markus Herderich for their skilled supervision. I would like to make special mention of my principal supervisor Dr. Patrick Ilard for his guidance, enthusiasm and insight.

The Grape and Wine Research and Development Corporation (GWRDC) and The Cooperative Research Centre for Viticulture (CRCV) for financial support.

The Nuriootpa Viticultural Research Station and Dr. Mike McCarthy for use of the trial site and providing the grape material.

Dr. Leigh Francis, Mr. Richard Gawel, Ms Kate Latte and the members of the panel for help and enthusiasm during the sensory trials.

Dr. Bryan Coombe and Dr. Peter Dry for constructive comments and encouragement.

Mr. Radimir Ristic for the technical assistance with the drawings of grape seeds.

Dr. Jim Kennedy, past and present members of the Tannin group for all the advice, help and encouragement.

The Department of Wine and Agriculture and The Australian Wine Research Institute, its administration, staff and students for providing resources, technical assistance and a stimulating work environment.

Dr. Robert Astenstorfer for motivation, help and friendship.

Ms Milica Grcic, Ms Sally Huston, Ms Freya Hohnen and Ms Mardi Longbottom for the technical assistance with grape material and winemaking.

My mother for all her help and encouragement.

Finally, I thank my family, Radimir, Denis and Marie for their patience, support and love.
LIST OF PUBLICATIONS ARISING FROM THIS THESIS

**Refereed Journals**


**Non-referred conference and related publication**


Chapter 1 – General introduction

The purpose of this introduction and literature review is to outline current understanding of some aspects of seed development in relation to berry development. The achievements of various studies investigating changes in phenolic composition of grape seeds and skins during berry development are highlighted. An understanding of the sequences of changes during seed and berry development and ripening is a critical foundation for a further research of the contribution of phenolic compounds to wine colour and sensory properties of wine. Different studies investigating the effect of sunlight intensity on the berry and/or phenolic compounds are also included.

1.1. Seed and berry development

1.1.1. Seed development

The grape berry may contain four seeds, having developed from four ovules within the ovary, but the number of seeds is usually less than four. The morphology of the grape seed (shape and structure) is a constant varietal characteristic (Viala and Vermorel 1910, Winkler and Williams 1936, Chadha and Randhawa 1974) while the number of seeds per berry and seed weight may vary with site, season and viticultural practice (Dragas et al. 1958, Hardie and Aggenbach 1996).

The shape of mature seeds of Vitis vinifera varieties is more or less pear-like or cuneate (Figure 1.1). There is a beak of variable length and by nature it may be straight or curved, rough, wrinkled or smooth. The beak also varies in thickness and the sharpness of the angle at the hilum. On the opposite end of the beak is a notch, as a small or larger indentation. The dorsal side is thick and rounded at the tip. A groove runs the length of the back, and around the centre forming a circular depression called the chalaza. The position of the chalaza in Vitis vinifera varieties is central or towards the notch. The chalaza may be circular or oval, distinct or not, raised or sunken. The ventral side has two sections called fossettes, seed folds or rumination ingrowths. Between the fossettes is a karina or keel in the form of a longitudinal ridge. The karina may be sharp or rounded and more or less prominent. A raphe extends from the hilum, along the ridge of
the keel and over through the notch to the dorsal side, where it ends with the chalaza (Figure 1.1).

![Diagram of grape seed](image)

Figure 1.1. Line diagram of dorsal and ventral sides of a mature grape seed showing the beak, hilum, notch, fossettes, karina, raphe and chalaza. Modified from Buric (1985).

Each seed develops and matures according to a defined pattern which begins with rapid cell division and cell enlargement. At the micropyle the outer layer thickens and forms the beak. Towards the chalaza, the middle layer of the outer integument becomes folded and produces the raphe with two depressions, called fossettes or rumination ingrowths on each side of the raphe. The development of rumination causes displacement of the chalaza and the seed enlarges considerably on its basal end. Meristematic activity in the three layers of the outer integument reaches a peak at 20-25 days after anthesis and then declines to nil at 45 days. As the endosperm grows, it replaces the nucellus (from 35 days after anthesis). The cells of the inner layer of the outer integument lignify and each of them contain a crystals except cells in the fossettes, thus this layer is thick at the beak and thin at the fossettes. At the same time, the inner integument remains thin and adheres to the endosperm (Pratt 1971, Mullins et al. 1992). The hardening of the integument is accompanied by changes of its colour.
In mature seeds the main roles of the seed coat are to protect the embryo and to control seed dormancy. It was indicated that this control might be based on the changes of the seed coat to reach a high degree of impermeability to water or oxygen (Werker 1980/81). To increase impermeability, the seed coat accumulates specific compounds such as crystals and phenolic compounds (flavan-3-ols and procyanidins). The localisation of these compounds in the grape seed coat has not been fully investigated although some studies have been reviewed by Pratt (1971). These studies have shown that in the seed coat there were: i) a papery outer layer with a thick cuticle, ii) a parenchymatous or a middle layer with cells containing monomeric flavan-3-ols (Thorngate and Singleton 1994) and iii) a hard layer that contains several layers of lignified cells (Fernandez and Sanchez-Gavito 1949) with groups of scleroids and crystal-bearing cells (Pratt 1971) and as well cells with procyanidins (Thorngate and Singleton 1994). Phenolic compounds and their derivatives have many functions. It has been suggested that phenolic compounds in the seed coat act as a biochemical barrier to the permeability to oxygen (Werker 1997). This is based on an assumption that oxidation of the phenolic compounds reduces the oxygen available to the embryo. Phenolic compounds are also responsible for seed coat colour. In seeds of *Pisum* species the correlations were established between the brown colour of the seed coat, the degree of impermeability of the seed coat and the presence of phenolics and quinones in the seed coat (Marbach and Mayer 1974, Werker et al. 1979).

1.1.2. Berry development
Various studies indicated that the number and weight of seeds are related to the berry weight and it’s ripening rate (Winkler and Williams 1936, Olmo 1946, Coombe 1960, Scienza et al. 1978, Cawthon and Morris 1982) and there is a wide support for the generalisation that seed development affects berry development.

Seed and berry growth and development occur simultaneously. Berry growth follows a double sigmoid curve pattern (Winkler and Williams 1936). The growth curve has been divided into three phases (Harris et al. 1968, Coombe 1973, Alleweldt et al. 1984), although some researchers designated two (Staudt et al. 1986) or four phases (Nitsch et al. 1960). Three periods in the course of growth are determined by using the parameters of the diameter, length and volume of berries and the weight of seeds. These parameters
differentiate phases of rapid growth, slow growth and final increase in growth. The duration of each growth period varies according to the characteristics of the vine and environmental conditions.

Period I is characterised by the rapid growth of the pericarp and seed, while the embryos remain small. In the pericarp there is rapid cell division lasting 3-4 weeks, followed by cell enlargement, firstly in the placenta and inner pericarp (7-11 days), later in the outer pericarp, and finally in the hypodermis and epidermis (32-40 days after anthesis) (Pratt 1971). By the end of this period, the seeds reach nearly their full size.

In period II (or lag phase) the growth rate of a berry is significantly slowed down. At the same time, there is a fast hardening of the seed endocarp. The embryo develops rapidly and reaches its maximum size around 70-75 days after anthesis (Staudt et al. 1986). Embryo growth shows no relation to the time of transition from the first to the second growth phase of the whole berry or to the double sigmodal growth (Staudt et al. 1986). At the end of period II the berries reach their highest level of acidity and begin to accumulate sugar. They also lose chlorophyll and the change of colour begins. Period II generally lasts 2-4 weeks, but in seedless varieties period II is significantly shorter.

Period III starts with veraison and lasts 5-8 weeks. This period is characterised by rapid cell enlargement and the growth of the berries. The texture of the berries is softer due to changes in the cell walls, anthocyanins accumulate in the skin which become coloured and the compounds involved in aroma and flavour develop. The berries accumulate glucose, fructose, total and free amino acids, proteins and nitrogen (Peynaud and Ribereau-Gayon 1971, Coombe 1973).

1.1.3. The influence of seeds on berry development and composition
Various studies have indicated that seed and berry growth and development occur simultaneously. The growth of a grape berry follows a double sigmodal curve pattern and consists of two phases of rapid growth separated by a phase of slow growth or lag phase. These phases are marked not only by changes in the diameter, length and volume of berries, but also by changes in the weight of seeds (Harris et al. 1968, Coombe 1973, Alleweldt et al. 1984). During the first phase of rapid growth the rate of pericarp cell
division was positively correlated with the growth rate of developing seeds (Coombe 1960, Coombe and McCarthy 2000). By the end of this phase the seeds reached nearly their full size but seed embryos still remained small (Coombe 1960, Staudt et al. 1986). The second phase of berry growth starts with veraison and it was reported that the beginning of veraison was associated with cessation of seed growth (Winkler and Williams 1936, Nakagawa and Nanjo 1965, Cawthon and Morris 1982). Growth of the seed embryo, at first very slow, became rapid and, depending on variety, reached its maximum size at 70-100 days after flowering (DAF) (Staudt et al. 1986, Ebadi et al. 1996). A study by Staudt (1986) showed that the maximum embryo length and maximum seed dry weight coincided with maximum berry weight.

The correlation between the number and weight of seeds and the size of fully ripened berries has been the subject of many studies (Winkler and Williams 1936, Bioletti 1938, Olmo 1946, Boselli et al. 1995, Ebadi et al. 1996). Olmo (1946) reported correlations between berry weight and seed weight (seed index number), the frequency and types of mature seeds and a correlation between seed number and berry weight. Although there are some indications that the relationship between seed and pericarp development is highly variable (Peynaud and Ribereau-Gayon 1971, Hardie and Aggenbach 1996), there is evidence that the number of seeds and the total weight of seeds in a berry are highly correlated to final berry size, fruit flesh mass and dry mass, and accumulation of $^{14}$C-photosynthate (Cawthon and Morris 1982, Ebadi et al. 1996, Ummarino and Di Stefano 1996). Other studies reported that the number of seeds affected acidity and total soluble solids (Cawthon and Morris 1982, Umarrino and Di Stefano 1996), the concentration of soluble sugars and the concentration of malic acid (Cawthon and Morris 1982) and the amount of skin and seed phenolics, as well as the compounds involved in wine aroma (Scienza et al. 1978, Ummarino and Di Stefano 1996, Ummarino and Di Stefano 1997).

Apart from the roles in seed and berry development, described above, seed tannins are claimed to provide health benefits, as they act as antioxidants (Ariga and Hamano 1990) and show antihepatoxic, anticarcinogenic, antihypertensive, antimutagenic, anti-inflammatory, antiviral and antiulcer properties (Saito et al. 1998).
1.2. Phenolic compounds in the grape berry

The phenolic compounds in the grape berry of interest for this study are the group of flavonoids which include flavonols (quercetin and quercetin-3-glucoside), anthocyanins and flavan-3-ols (catechin, epicatechin, epicatechin gallate and epigallocatechin). In grape skins and seeds flavan-3-ols may exist as monomers or as terminal and extension subunits of tannins.

Flavonoids are generally biosynthesised by the shikimate pathway, from which they are produced using intermediates of carbohydrate metabolism. A schematic diagram of the general phenylpropanol and flavonoid biosynthesis pathway is given in Figure 1.2. There are many enzymes involved in flavonoid biosynthesis and they may be divided into two groups: a group of enzymes that catalyse the general phenylpropanol metabolism and a group of specific enzymes for the synthesis of flavonoids (Boss *et al.* 1996, Haslam 1998). These studies indicated that the two groups of enzymes respond differently to environmental conditions, such as light (Haslam 1998).

![Figure 1.2. A schematic diagram of the general flavonoid biosynthesis.](image-url)
Although all flavonoids share the same pathway, there is little evidence in the literature about the relationship between the anthocyanin biosynthetic pathway and the metabolism of tannins. Flavonols are synthesised in the upper epidermal cells of berry skin, while anthocyanins are synthesised in the lower epidermis and both accumulate in the central vacuole (Moskowitz and Hrazdina 1981). There is evidence about multiple activities of the enzyme glucosyltransferase which suggested that anthocyanin and flavonol glucosylation reactions are catalysed in vivo by different enzymes (Ford and Hoj 1998).

1.2.1. Monomeric flavan-3-ols and tannins

1.2.1.1. Monomeric flavan-3-ols and tannins in the grape seed
Flavan-3-ols in the grape seed are: catechin (C), epicatechin (EC) and a galloylated form, epicatechin-gallate (ECG) (Figure 1.3). Procyanidins (tannins) are formed in the oligomerisation process and comprised of a flavan-3-ol terminal subunit and extension subunits connected by the interflavan linkages (C₄-C₈ or C₄-C₆) (Delcour et al. 1983, Haslam 1998) (Figure 1.4). The majority of the interflavan bonds comprise the C₄-C₈ type, while a minority have C₄-C₆ orientation (Haslam 1998).

Figure 1.3. Structure of flavan-3-ols: epicatechin, catechin, and epicatechin gallate.
Figure 1.4. General structure of tannins formed from flavan-3-ols through C4-C8 or C4-C6 bond and a general structure of a trimer. Modified from Fuleki and Ricardo da Silva (1997).

Many studies have investigated the composition and concentration of monomers, dimers and trimers in grape seeds of different varieties (Czochanska et al. 1979, Ricardo da Silva et al. 1991a, 1991b, Ricardo da Silva et al. 1992, Santos-Buelga et al. 1994, Katalinic and Males 1997, Saint-Cricq de Gaulejac et al. 1997, de Freitas and Glories 1999, Jordao et al. 2001, Mateus et al. 2001, Mateus et al. 2002a). The content of monomers have been found to increase from a very low level in the early stages of seed and berry development, to a maximum around veraison, and thereafter sharply decreased. Dimers linked through C4-C8 such as procyanidin B3 were the most abundant in unripe berries, B2-gallate at veraison and B2 at maturation. Similar changes
were observed for several varieties from the Douro Valley (Mateus et al. 2001), Semillon and Ugni Blanc (de Freitas and Glories 1999) and Castelao Frances and Touriga Francesa (Jordao et al. 2001). Studying a clone of Merlot Noir, Saint-Cricq de Gaulejac et al. (1997) observed that the accumulation of dimers B1, B4, B7 and B8 made up of catechin and epicatechin units decreased more rapidly during ripening, than dimers or trimers constituted only from epicatechin units (B2, B5, A2 and C1). There was a significant correlation between the concentrations of procyanidins with a C₄-C₈ bond, namely A2, B2 and C1. On the contrary, no relation between the concentrations of procyanidins with C₄-C₈ and procyanidins with C₄-C₆ linkages has been reported, suggesting that there are two different biochemical pathways for the formation of these types of dimers. However, it is believed that seeds with a higher percentage of a monomer epicatechin or catechin, show higher relative proportions of oligomers or tannins containing this compound in the terminal unit (Haslam 1977, Stafford and Lester 1985, Santos-Buelga et al. 1994). As monomers and oligomers (dimeric and trimeric) represent less than 10% of total tannin concentration in seeds (de Freitas and Glories 1999), it became necessary to investigate the composition of tannin polymers consisting of more than three or four subunits. Thus the composition of seed tannins and their changes during seed and berry development have been studied more intensively in recent years (Kennedy et al. 2000a, Kennedy et al. 2000b, Peyrot des Gachons and Kennedy 2003, Downey et al. 2003a). The general trend of developmental changes in the studied varieties of Cabernet Sauvignon, Shiraz and Pinot Noir showed that the bulk synthesis of tannins in grape seeds occurred in the green berries, with the maximum amount reached around veraison, and after that, the amount dropped rapidly and remained relatively constant during the maturation period. However, some studies also reported that the concentration and composition of seed tannin may be influenced by variety and season (Oszmianski and Sapis 1989, Ricardo de Silva et al. 1991d, Ricardo de Silva et al. 1992, Katalinic and Males 1997, de Freitas et al. 1999). While some varieties such as Cabernet Sauvignon, Shiraz and Merlot have naturally low levels of seed tannins, others, such as Cabernet Franc, Pinot Noir, Grenache and Tempranillo have much higher levels (Ribereau-Gayon et al. 2000).
1.2.1.2. Monomeric flavan-3-ols and tannins in the berry skin
Flavan-3-ols in the berry skin are represented by catechin (C), epicatechin (EC), epicatechin gallate (ECG) and epigallocatechin (EGC). The main differences between the composition of skin and seed tannins are that skin tannins have lower proportion of galloylated units and higher average molecular weight (mDP). Another difference is that epigallocatechin units are only found in skins (Souquet et al. 1996, Cheynier et al. 1997). The localisation of tannins in the berry skin, as described by Amrani Joutei et al. (1994) may be: i) in the vacuoles, forming dense clusters in the cells close to the epidermis and diffuse granulations in the internal cells of the mesocarp, ii) tannins bonded strongly to the proteophospholipidic membrane (tonoplast) and iii) tannins integrated in the cellulose-pectin wall.

In skins of mature berries of Merlot and Cabernet Sauvignon the most abundant component was catechin, and dimer B2, followed by trimer C1 (de Freitas et al. 2000). While procyanidin B2 have been found in the majority of red varieties, it is absent in white varieties where it is replaced by dimer B1 (Ricardo de Silva et al. 1991d, de Freitas and Glories 1999, Ribereau-Gayon et al. 2000). Monomers appeared prior to veraison and then decreased to a constant level. Other studies have shown that the concentration of skin tannins is relatively high at fruit set (Downey et al. 2003a) after which it declined and then increased again to a maximum observed around veraison, followed by another decrease (de Freitas et al. 1999, Kennedy et al. 2001a, Kennedy et al. 2002, Downey et al. 2003a).

1.2.2. Anthocyanins
The major anthocyanins in Vitis Vinifera varieties are derived from aglycones of cyanidin, delphinidin, malvidin, peonidin and petunidin (Figure 1.5). These structures may be present as mono-glucosides or acylated heterosides. The acylated anthocyanins have an organic acid (either acetic acid or coumaric acid) esterified with the hydroxyl on the six carbon of a glucose moiety. Only mono-glucoside anthocyanins and their acetate and coumaroyl derivatives have been identified in Vitis Vinifera grapes and wines (Ribereau-Gayon et al. 2000).
Accumulation of anthocyanins begins at veraison and consists of the three phases: i) a slow increase, ii) a rapid increase and iii) a decrease at the end of ripening (Hrazdina et al. 1984). Initially, only the dihydroxylated anthocyanins, cyanidin and peonidin accumulate, then formation of trihydroxylated anthocyanin, delphinidin, petunidin and peonidin occur (Mazza 1995, Boss et al. 1996, Keller and Hrazdina 1998). Several researchers (Somers 1976, Roggero et al. 1986, Keller and Hrazdina 1998) have observed a decrease in the concentration of anthocyanins at the end of ripening which could be due to the breakdown of anthocyanins by glucosidase and peroxidase activity in the grape skin vacuole (Keller and Hrazdina 1998). In Shiraz berries the majority of the anthocyanin pool is represented by malvidin and its derivatives (Boss et al. 1996, Haselgrove et al. 2000). Many studies showed that the anthocyanin composition and the pattern of development of anthocyanins are strongly influenced by cultivar, season, location and viticultural practices, as reviewed by Jackson and Lombard (1993).

1.2.3. Flavonols
Quercetin which may also be present as quercetin-3-glucoside and quercetin-3-glucuronide is the main flavonol in the grape berry skin (Cheynier and Rigaud 1986, Price et al. 1995a). The structure of quercetin is given in Figure 1.6. Similar to other phenolic compounds, high concentration of quercetin has been recorded around fruit set,
followed at first by a decrease, and then remained at a constant level (Keller and Hrazdina 1998, Haselgrove et al. 2000, Downey et al. 2003b).

![Figure 1.6. Structure of quercetin.](image)

The major role of flavonol quercetin appears to be in UV protection by absorbing light in the 280-320nm region and acting as an UV filter (Flint et al. 1985). It has also been indicated that it may play a role in plant development and plant defence against pathogens and environmental conditions (Harborne and Williams 2000).

### 1.3. Influence of sunlight intensity on berry composition

#### 1.3.1. Influence of sunlight intensity on berry weight, total soluble solids, juice pH and titratable acidity (TA)

The effects of sunlight intensity on berry composition are complex. Sunlight influences fruit composition through: a) photosynthetic effects by providing the light energy for photosynthesis and other light-stimulated metabolic process, and b) phytochrome and thermal effects by providing direct solar radiation on plant surface or by heating the surrounding air (Crippen and Morrison 1986b). Temperature and solar radiation are linearly related and it is difficult to separate their effects on fruit composition (Smart 1987).

Leaf shading and bunch shading, whether natural or artificial, have been shown to have different effects on grape berry composition (Crippen and Morrison 1986a, Rojas-Lara and Morrison 1989, Morrison and Noble 1990, Gao and Cahoon 1994). While in some experiments berry weight of shaded berries was greater than that of exposed berries (Crippen and Morrison 1986a, Reynolds et al. 1986, Blanke and Leyhe 1987, Price et
al. 1995c) other studies reported a higher berry weight of exposed berries (Dokoozlian and Kliewer 1996). A greater berry weight of shaded berries was related to a lower berry temperature which usually results in lower transpiration rate and higher turgor pressure and thus greater berry weight (Blanke and Layhe 1987). In contrast, a lower berry weight of shaded berries was associated with limited photosynthesis and carbohydrate transport (Sepulveda et al. 1986, During and Davtyan 2002). Archer and Strauss (1989) and Cartechini and Pallioti (1995) observed a decreased in berry mass, bunch mass and yield per vine as a result of canopy shading of Cabernet Sauvignon and Sangiovese respectively. Crippen and Morrison (1986) and Price (1994) reported that moderately exposed bunches were bigger than shaded and highly exposed bunches. In these experiments a lower weight from shaded bunches was related to fewer berries, whereas a lower weight of exposed clusters resulted from smaller berries. On contrary, Gao and Cahoon (1994) and Keller et al. (1998) did not observe any effect of shading on bunch weight of Reliance and Cabernet Sauvignon respectively.

Generally, canopy shading delays sugar accumulation and ripening, while bunch shading had little effect on these phenomena (Smart 1985). In shaded leaves, whether shading was achieved naturally or artificially, photosynthesis was compromised and fruit from shaded treatments had lower sugar content (Kliewer and Lider 1970, Reynolds et al. 1986, Rojas-Lara and Morrison 1989, Keller et al. 1998, Mabrouk and Sinoquet 1998). Iland (1989b) showed that the effect of berry shading and/or leaf shading on juice pH and TA may vary depending on which factor is dominant. In the varieties Seyval Blanc and Cabernet Sauvignon shaded leaves resulted in higher malic acid level, pH and titratable acidity in the berry juice (Reynolds et al 1986, Morrison and Noble 1990). Extensive shading of Shiraz vines resulted in berries with higher pH and lower titratable acidity (Smart et al. 1985a). Other studies reported high positive correlation between potassium content, on one side and the malate concentration and juice pH on the other (Hale and Buttrose 1973, Smart et al. 1985b, Rojas-Lara and Morrison 1989).
1.3.2. Influence of sunlight intensity on phenolic composition of berries

Berry exposure to sunlight has both quantitative and qualitative effects on phenolic compounds in grape skins. Increased light exposure correlated with increased total phenol content of berries (Kliewer 1977) whilst high temperature was negatively correlated with total berry phenols (Singleton and Nelson 1966, Buttrose 1970, Pirie and Mullins 1977). Many studies have shown that both temperature and sunlight have a significant effect on anthocyanin synthesis. The summary of the combined effects of light and temperature on anthocyanin production as suggested by Iland (1989b) is given in Figure 1.7.

**SHADED LEAVES**

- Decreased photosynthesis due to decreased light interception, and therefore less substrates (sugars) for anthocyanin synthesis in the berry

**SHADED BERRIES**

- Decreased berry anthocyanin production due to decreased light interception (B) combined with:
  - Decreased anthocyanin production if berry temp. not in optimum range (C)
  - Increased anthocyanin production if berry temp. in optimum range (D)

**Overall effect on berry anthocyanin production**

- Decreased anthocyanin production if effects of A, B, and C combine
- Decreased anthocyanin production if effects of A and B dominate the effect of D
- Increased anthocyanin production if effect of D dominates effects of A and B

Figure 1.7. Effect of canopy density on berry anthocyanin production. Adapted from Iland (1989b).
Optimum temperature range for anthocyanin synthesis is 17-26°C (Pirie and Mullins 1977). Sun exposure increases the solar heating of the fruit, and thus heating has been associated with the reduction in the content of anthocyanins. Prolonged periods at day/night temperature of 37/32°C under high light intensity permanently blocks or inactivates the enzyme systems for anthocyanin synthesis (Kliewer 1977). Mabrouk and Sinoquet (1998) showed that the concentration of anthocyanins and phenolics in grapes are related to the direct effect of light on leaves and to interactions between light and temperature effects on bunch.

Extensive shading with 85% or 95% shade cloths significantly reduced anthocyanins accumulation in berries of Emperior (Kliewer 1977) and Reliance (Gao and Cahoon 1994) respectively, while the concentration of anthocyanins in berries that were developed and ripened under 46% shading or 55% shading was similar to those of fully exposed berries. Decreased anthocyanin concentration has been reported in experiments using artificial (Kataoka et al. 1983, Rojas-Lara and Morrison 1989) or natural shading (Crippen and Morrison 1986b, Morrison and Noble 1990). Other studies have shown that the accumulation of anthocyanins in fully exposed berries may be similar to those in berries grown under relatively low sunlight intensity such as 22% of ambient (1900 PPFD) for Malbec grapes (Kliewer 1977), less than 20% of ambient (<260 PPFD) for Cabernet Sauvignon (Keller and Hrazdina 1998), less than 18% of ambient (<400 PPFD) for greenhouse-grown Pinot Noir (Dokoozlian 1990) and 10% of ambient (PPFD not reported) for Merlot (Mabrouk and Sinoquet 1998).

The high temperature effect on anthocyanin accumulation was observed in the study of Mabrouk and Sinoquet (1998) as the highest level of anthocyanins was found in moderate exposed bunches. A lower accumulation of anthocyanins observed in berries of Touriga National and Touriga Francesa that were grown in vineyards situated at the low altitude was also associated with higher temperature conditions compared to berries from vineyards at the higher altitude (Mateus et al. 2002a). However, it was suggested that the temperature effect may vary with cultivar, eg. anthocyanin biosynthesis was more sensitive to high temperature in Cardinal berries than Pinot Noir (Spayd et al. 2002).
Sensitivity of anthocyanin metabolism to sunlight intensity varies between individual anthocyanins. No or little anthocyanins were synthesised in the skins of Tokay, Emperior and Cardinal when they were developed and ripened in bags (Weaver and McCune 1960, Wicks et al. 1982). This was associated with high light sensitivity of cyanidin and peonidin biosynthesis to sunlight, as those varieties have cyanidin and peonidin as dominant anthocyanins (Kliwer 1977, Wicks et al. 1982). High demand for sunlight of cyanidin and peonidin biosynthesis was also observed in studies of Iacono et al. (1995) and Keller and Hrazdina (1998). In berries of Reliance, 95% of cluster shading decreased delphinidin and cyanidin mono-glucoside while peonidin and malvidin mono-glucoside and acylated cyanidin derivatives increased (Gao and Cahoon 1994). Bunch shading of Shiraz enhanced accumulation of malvidin coumaroyl-glucoside relative to mono-glucoside (Haselgrove et al. 2000). The accumulation of flavonols, in particular quercetin-3-glucoside, significantly increased with bunches exposure (Price 1994, Price et al. 1995a, Haselgrove et al. 2000).

1.4. Phenolic compounds in wines
Phenolic compounds in wines originate from skins and seeds and they essentially contribute to the colour of wine (intensity and stability) and taste (astringency and bitterness) (Jurd and Somers 1970, Haslam 1980, Dallas et al. 1995, Ricardo de Silva 1997, Cheynier et al. 2000). Only about 40% or less of the total phenol content in grape berries is recovered in young wine with about 60% lost by incomplete extraction, adsorption or precipitation with solids (pomace, yeast, and proteins), conversion to non-phenolic products or polymerisation to form insoluble precipitates (Singleton and Trousdale 1992). The phenolic compounds are extremely reactive and many of them are substrates for various enzymes, including esterases, glycosidase and oxidases (Cheynier et al. 1997). Their reactions commence with the crushing of the grapes and continue through maceration and fermentation operations. This produces a great diversity of products which contribute to the complexity of wine composition.

During winemaking and wine maturation tannins undergo structural changes for which two reactions are responsible: i) the acid-catalysed rupture of the interflavan bond leading to a complex bond-breaking and bond-making process and formation of polymerised structures which may precipitate (Timberlake and Bridle 1976, Haslam
1980) and ii) the facile electrophilic substitution of the "A" ring of the flavan-3-ol monomeric unit which yields oligomeric structures. Other reactions include oxidation reactions which lead to browning (Oszmianski et al. 1985, Cheynier et al. 1991, Cheynier et al. 1997, Yokotsuka and Singleton 2001).

The presence of acetaldehyde, which is usually found in wines as a result of yeast metabolism or ethanol oxidation, leads to condensation products of tannin-tannin and anthocyanin-tannin polymers (Fulcrand et al. 1996, 1997, Saucier et al. 1997a, 1997b, Es-Safi et al. 1999). The reaction starts with protonation of acetaldehyde, followed by nucleophilic addition of the resulting carbocation to yield an intermediate polyphenol-ethanol adduct (Timberlake et al. 1976, Cheynier et al. 2002). The ethanol adduct looses a water molecule to give a new carbocation intermediate which is in turn attacked either by another flavanol unit to give ethyl-linked tannin-tannin adduct (T-T) or by an anthocyanin to give a flavanol-ethyl-anthocyanin adduct (T-A) (Cheynier et al. 1997) (Figure 1.8). If anthocyanins are terminal units, they end the polycondensation reaction, whereas flavanols maintain the polymerisation process (Es-Safi et al. 2002). It was also shown that the acetaldehyde-induced condensation may occur between procyanidins and anthocyanins (Dallas et al. 1996a, Dallas et al. 1996b). Reactions between anthocyanins and tannins could occur directly, but at much slower rate (Jurd 1969, Somers 1971, Timberlake and Bridle 1977, Baranowski and Nagel 1983, Liao et al. 1992, Bakker et al. 1993, Garcia-Viguera et al. 1994). These reactions produce a colourless bicyclic A-T adducts, which has been reported to oxidise to the corresponding flavyium A*-T adducts (Remi et al. 2000, Cheynier et al. 2002) (Figure 1.7). Besides, it can rearrange to a yellow xanthylum salt (Timberlake and Bridle 1976, Liao et al. 1992).
Figure 1.8. Hypothetical mechanisms leading to tannin-tannin and tannin-anthocyanin additions with and without the presence of acetaldehyde. Modified from Cheynier et al. (2000).
In recent years many new pigments have been characterised as a result of the reactions between anthocyanins and small molecules such as pyruvic acid (Bakker and Timberlake 1997, Fulcrand et al. 1998, Romero and Bakker 1999), p-vinylphenol (Cameira-dos-Santos et al. 1996, Fulcrand et al. 1996), 4-vinylguaiacol (Hayasaka and Asenstorfer 2002), vinyl-flavanol (Asenstorfer et al. 2001, Mateus et al. 2003). Other studies have reported pigments resulting from the reaction between anthocyanins and flavan-3-ols directly (Santos-Buelga et al. 1995, Remi et al. 2000) or with the presence of acetaldehyde (Timberlake and Bridle 1976, Baranowski et al. 1983, Rivas-Gonzalo et al. 1995, Dallas et al. 1996a, Es-Safi et al. 1999, Mateus et al. 2002b). It has been shown that these pigments due to their structural properties are very stable, resistant to colour bleaching by sulphur dioxide and contribute to orange-red colour of wines (Bakker and Timberlake 1997, Mateus and de Freitas 2001, Vivar-Quintana et al. 2002).

The formation of a variety of pigmented and non-pigmented polymers during wine fermentation and maturation is via a chemical bond between phenolic compounds. Due to a large size and complexity of a variety of pigmented polymers their isolation and structural elucidation is difficult. Copigmentation is considered as an intermediate interaction in the polymerisation process between compounds which does not involve chemical bond formation (Liao et al. 1992, Brouillard and Dangles 1994, Boulton 2001, Lambert 2002). Copigmentation involves self-association of anthocyanins, intramolecular copigmentation and intermolecular copigmentation. The mechanism of self-association involves hydrophobic interactions between the aromatic nuclei of the anthocyanin with the hydrophilic glucose substituent surrounding it in an appropriate spatial arrangement (Hoshino et al. 1980). Intramolecular copigmentation involves interaction between aromatic pigment moiety and a copigment which are part of the same molecule (Goto and Kondo 1991). It is believed that intramolecular copigmentation between the aromatic \( p \)-coumaric acid substituent on the glucose moiety with the aromatic centres stabilises the anthocyanin from hydration. Intramolecular copigmentation represents an association of an anthocyanin with a copigment and depends on the chemical structure of anthocyanins (Brouillard 1983), nature of copigment (Asen et al. 1972, Baranac et al. 1996, Lambert 2002) and their concentrations (Davies and Mazza 1993). The model studies of copigmentation enhanced knowledge about possible reactions between anthocyanins and different
copigments (including tannins) and their effects on wine colour, but they generally used larger concentrations of cofactor to pigment than would be formed in wines (Mazza and Brouillard 1987, Liao et al. 1992, Davies et al. 1993, Baranac et al. 1996, Lambert 2002). However, these studies showed that the colour response to an increase of the ratio of cofactor to pigment was linear for any studied pair of anthocyanin and cofactor. Boulton (2001) hypothesised that copigmentation in wines may include a competitive equilibrium involving anthocyanins and many cofactors and may depend on their concentrations and solubility. During fermentation an equilibrium based on adsorption-desorption is established between the anthocyanins concentration in berries and in the wines. According to Boulton (2001) a role of a cofactor is to capture free anthocyanins, thus more could be extracted from berries into wines, which would ultimately lead to greater formation of polymeric pigments.

It is believed that skin tannins due to their localisation in vacuolar liquid, bound to vacuolar membrane and the cell wall, are more easily extracted during winemaking than seed tannins (Singleton and Draper 1966, Amrani Joutei et al. 1994, Thorngate and Singleton 1994, Sun et al. 1999, Peyrot des Gachons and Kennedy 2003). Various studies have shown that a higher rate of tannin polymerisation and copolymerisation between anthocyanins and tannins are dependent on their concentration and composition (Baranowski and Nagel 1983, Bakker et al. 1993, Picinelli et al. 1994, Dallas et al. 1996b, Cheynier et al. 2000, Romero and Bakker 2000). It was indicated that if there is a balance between the concentrations of anthocyanins, seed and skin tannins this may lead to the greater formation of stable pigmented and non-pigmented polymers and ultimately to a higher wine quality (Singleton 1992, Cheynier et al. 1998, Brossaud et al. 1999). However, the rate of extraction and diffusion of the phenolic compounds from berries during fermentation of grapes depends not only on the total phenolic content in grape berries and but also on winemaking techniques such as maceration time, temperature and levels of alcohol and SO₂ (Ricardo da Silva et al. 1993, Sun et al. 2001).

Red wine colour density is commonly measured as the sum of the absorbance at 520 nm and 420 nm. Wine colour density of red wines is derived from monomeric anthocyanins, oligomeric and polymeric pigments in equilibrium with anthocyanins, and tannins (Ribereau-Gayon 1982). In wines, the anthocyanins exist in equilibrium of
seven different structures including the flavylium ion, the quinonidal base, the water adduct, two isomers of the hemiketal and cis- and trans-chalcone (Brouillard and Delaporte 1977). This equilibrium is dependent on pH and may be affected by SO₂. In very acidic solutions, anthocyanins exist in the highly coloured flavylium form, while at wine pH, they exist in an equilibrium of coloured (quinoidal base) and colourless form (hemiketal and chalcone structures) (Asenstorfer 2001). Oligomeric and polymeric pigments are relatively resistant to change in pH and are not bleached by SO₂ (Fulcrand et al. 1996, Bakker and Timberlake 1997, Asenstorfer 2001). Wine colour is an important aspect of sensory properties of wines and a correlation between wine colour density and wine quality has been demonstrated in several studies (Somers 1972, Somers and Evans 1975, Jackson et al. 1978, Ribereau-Gayon 1982).

1.5. Phenolic compounds in wines and wine sensory properties
Astringency is a tactile sensation in which a drying, puckering and roughing feeling is produced by the interaction of wine tannins with salivary proteins (Robichaud and Noble 1990). The interaction consists of both hydrophobic interactions and hydrogen bonding and is influenced by the degree of polymerisation, galloylation and hydroxylation of the tannins (Gawel 1998, Peleg et al. 1999, Sarni-Manchado et al. 1999b, Vidal et al. 2003b). Bitterness is a taste sensation, most effectively elicited at the back of the tongue, which increases with increasing concentrations of flavan-3-ol monomers (Haslam 1998).

The tannins are largely responsible for the astringency of red wine, whilst the monomers contribute significantly to its bitterness. As their concentration increases, the maximum intensity and duration of perception of both bitterness and astringency increase. The high concentration of relatively low molecular weight phenols, such as catechin, epicatechin and epicatechin-gallate may give rise to an astringent response (Haslam 1998). The stereochemistry of the monomeric flavan-3-ol may also affect bitterness and astringency. Thus epicatechin was more astringent and bitter than catechin, and its astringency and bitterness were more persistent at all concentrations (Thorngate and Noble 1995). The higher intensity of bitterness of epicatechin was related to its greater lipophilicity, since epicatechin shows lower solubility in water and later elution from reverse-phase HPLC column (Gardner 1979). Studying astringency
and bitterness of seven flavonoid compounds Peleg et al. (1999) reported that dimers with C₄-C₆ bond were more bitter than dimers with C₄-C₈ bond and the most astringent compound was B₆ dimer.

Astringency and bitterness are influenced by the degree of polymerisation. As the molecular size increases, bitterness has been found to decrease and astringency increase. Bitterness is elicited by interaction with a specific bitter membrane-bound receptor or through surface membrane interaction. Thus the increase of the molecular size of procyanidins, decreases bitterness by limiting the access to membrane-bound receptor or by direct depolarisation of the taste receptor cell (Peleg et al. 1999). It is believed that the increase in perceived astringency with the degree of polymerisation is due to greater capacity of polyphenols to bind the proteins and hence elicit astringency (Lea and Arnold 1978). The longer and more hydrophobic polyphenols bind more strongly to the proline-rich peptide and they are predominantly precipitated together with the salivary proteins (Sarni-Manchado et al. 1999a). Baxter et al. (1997) proposed a model in which multiple polyphenol/polyphenol and polyphenol/PRP interactions act cooperatively to produce a high-molecular mass aggregate, which precipitate and generate the astringency. Galloylation may also enhance astringency, by increasing tannin interactions with various proteins (Cheynier et al. 1998).

Recently Vidal et al. (2002) reported that seed tannins are more astringent, coarse and drying than skin tannins of equivalent size which may be related to gallic acid derivatives. The study also supported the finding that increased concentration of tannins enhanced the astringency related mouth feel-attributes. Partially purified anthocyanin fraction in a model wine was described as slightly astringent (Brossaud et al. 2001, Vidal et al. 2002), but when added to a solution containing seed tannins, the astringency increased (Brossaud et al. 2001). It was also indicated that anthocyanins may improve wine flavour (Singleton and Noble 1976) and enhance perception of wine fullness (Vidal et al. 2002).

Although the intensity and total duration of perceived astringent and bitter sensations are influenced by the concentration and composition of phenolic compounds in wines, some other wine properties may influence the taste sensation by altering the salivary flow and composition of the individual’s saliva. The wine pH, ion concentration,
temperature and ethanol concentration affect the perceived astringency and bitterness by influencing phenol-salivary protein binding and by directly reducing salivary viscosity. The major influence is that of ethanol concentration and the intensity of bitterness increased as the ethanol concentration increased (Fischer and Noble 1994, Noble 1998), which may be associated with “hardening” of tannin perception (Noble 1990). Furthermore, low pH and/or higher acidity in model wines seem to increase the intensity and duration of astringency (Guinard et al. 1986a, 1986b, Fischer and Noble 1994, Kallithraka et al. 2001).

In summary, the sensory properties of red wines are largely related to the phenolic composition of grape skin and seeds. Skin and seed tannins have same structural properties and distinct organoleptic properties. During fermentation and ageing of a wine a number of reactions occur between anthocyanins and tannins, resulting in products that lead to the greater complexity of the sensory characters. These reactions result in colour changes and increased colour stability. Bitterness and astringency, and their balance are dependent on the tannin concentration and structure (the degree of polymerisation and galloylation), and may be affected by some other constituents, such as tannin-anthocyanin adducts.

Canopy shading produces differences in the aromas of both fruit and wine. Extensive shading may result in lower concentration of quality enhancing aroma compounds, while over-exposure to sunlight lead to unacceptably high concentration of phenolics and some undesirable compounds (Smart et al. 1985). Sensory evaluation of juice and wine of Cabernet Sauvignon, from vines with different levels of sun exposed clusters indicated that panellists could detect some differences in aroma and flavour of wines (Morrison and Noble 1990). In other studies wine quality differences were small, but wines from exposed fruit were scored higher, which was probably due to darker colour and more intense aroma (Reynolds et al. 1986, Archer and Strauss 1989, Price et al. 1995a).
1.6. Canopy manipulation and vine canopy microclimate

Vineyard management techniques to manipulate the light intensity at the bunch zone have been the subject of extensive research aiming to create an optimal canopy microclimate which is conductive to the production of high quality grapes. The modifications of vine canopy change the canopy microclimate which influence either directly or indirectly fruit composition (Kliewer 1970, Smart et al. 1985a, 1985b, Dokoozlian and Kliwe ar 1995a, 1995b, Kliwe ar and Dokoozlian 2000). Microclimate is essentially dependent on vine density; the amount of foliage and the way that foliage is displayed Smart and Robinson (1991). The assessment of various parameters of canopy microclimate may determine the differences in vine canopy density and conditions under which grapes are grown and ripen.

Solar radiation and air temperature are the major elements of the canopy microclimate. Vine leaves strongly absorb red solar radiation in the wavelength range from 400 to 700 nm (called photosynthetically active radiation (PAR), measured as photosynthetic photon flux density (PPFD)). Usually 80-90% of PAR is absorbed at the surface of green leaf while the reminder of 10-20% is transmitted or reflected. At the same time, leaves reflect or transmit the near infrared radiation in the wavelength beyond 700 nm (Smart 1985). The red light (660 nm) converts the inactive phytochrome form to active, but the reaction is reversible by far-red light (730 nm) or high temperature conditions. Thus the ratio of red: far red (R:FR) regulates the photo-equilibrium of photochrome. Photochrome is known as one of the photoreceptor responsible for the detection of light quality by plants. It has been shown that photochrome influences many aspects of plant growth and metabolism and has been implicated in the regulation of berry growth and composition Smart et al. (1988). Another important aspect of the canopy light environment is the frequency and duration of sunflecks which occur when sunlight enters gaps in the canopy. Pearcy (1990) has shown that sunflecks have a significant effect on the carbon translocation in leaves located in the canopy interior.

Sunlight influences canopy microclimate by heating the surrounding air. Heat from sunlight may influence reaction rates of metabolic processes and can cause stress either by direct temperature stress or dehydration (Crippen and Morrison 1986a). Air temperature is one of the main factors that affect translocation and distribution of organic and inorganic substances in plants (Sepulveda et al. 1986). The temperature
effects are complex because they incorporate effects of other components such as a convective heat loss, wind velocity, tissue thermal conductivity, fruit transpiration and thermal exchange by radiation. Temperature and solar radiation are linearly related thus it is difficult to separate their effects on plant metabolism (Smart 1987).

The canopy microclimate is essentially dependent on the canopy density. The level of canopy density depends on shoot spacing, orientation, length and shoot vigour (Smart 1989). A dense canopy is defined as a canopy with most leaves and fruit in shade. In dense canopies younger leaves are usually located at the exterior, while older basal leaves and bunches are in intense shade and they experience lower day-time and higher night-time temperatures, lower levels of PAR and shortwave radiation, decreased values of radiation ratio R:FR, higher relative humidity and lower wind velocity (Smart 1980). Dense canopies had a low level of PAR (1% of ambient) and a decreased ratio of R:FR (0.07-0.31, which is approximately 10% of ambient) (Smart et al. 1985a, Dokozilian and Kliwer 1995a). In dense canopies direct sunlight is usually absorbed at the canopy surface and only 30% penetrates as sunflecks (Smart 1973). Frequency and duration of sunflecks in the interior of dense canopies was reported as 0.6s\(^{-1}\) and 1.2s by Kriedemann (1968). Strong correlations have been established between PAR and the ratio R:FR \((r=0.98)\) and between PAR and sunflecks \((r=0.896)\) for canopies of different levels of density (Dokoozlian and Kliwer 1995b).

Shaded leaves may be below air temperature due to transpiration cooling (Smart et al. 1985a). Millar (1972) did not find differences between shaded and exposed leaf temperature of Carignane, Muscat of Alexandria and Torontel varieties grown in Chile, while the difference in berry temperature for exposed and shaded berries was 1.4 to 7.3 °C higher and 0.5 to 4.4 °C lower than air temperature, respectively. On the contrary, excessive exposure to solar radiation and high temperature increased berry temperature up to 10.5°C above air temperature as was in a case of Thompson Seedless cultivars grown in California (Tarara et al. 2000). In the centre of dense canopies wind velocity is usually reduced by 20%, humidity is increased (up to 10%) and evaporation reduced due to lower levels of radiation, wind speed and increased humidity (Smart 1980).

Smart and Robinson (1991) introduced 8 indicators rated by a 10-point system to describe characteristics of vine canopies and hence the difference between them. Three
characteristics relate to the canopy microclimate (canopy gaps, canopy density and fruit exposure) and five to the physiological status (leaf size, leaf colour, shoot length, lateral growth and presence of active shoot tips). The optimum values of the indicators are dependent upon specific varieties in certain climates (Smart and Robinson 1991, Dokoozlian and Kliewer 1995b) and they can be used for the assessment of the differences between canopies such as canopy density, shoot vigour and bunch and leaf exposure to sunlight.

1.7. Aims of the study
The literature review highlighted a need to gain a greater knowledge and understanding of the pattern and sequences of biological and chemical changes during seed and berry development. As phenolic compounds from seeds and skins contribute to wine colour and sensory properties of wines, there is a necessity to further investigate the link between berry composition and wine composition and sensory properties of wine. Viticultural practices may affect phenolic composition of berries and while it has been documented that canopy shading, whether natural or artificial, affects the chemical composition of berries, it has not been established whether different levels of sunlight intensity at the bunch zone may affect seed phenolic composition, and hence, potentially the chemical and sensory properties of wines.

The aims of this study were:

- to describe changes in the morphological characteristics of seeds during berry development,
- to determine relationships between morphological characteristics and phenolic composition of seeds,
- to determine relationships between seed and skin phenolic compounds,
- to determine the effects of the intensity of sunlight interception at the bunch zone on phenolic compounds of seeds and skins,
- to determine the effects of the intensity of sunlight interception at the bunch zone on phenolic composition and sensory properties of wines, and
- to further investigate the link between berry composition, wine composition and sensory properties of wine.
The following chapters describe experimental procedures of several investigations undertaken in this study. They were designed to increase understanding of some aspects of biological and chemical changes during seed and berry development, and relationships between them in relation to their contribution to wine composition. This study also emphasises the need to measure and express the amount of investigated compounds as a level (on a per berry basis) and/or as a concentration (on a per gram berry weight basis).
Chapter 2 – Experimental design and vine characteristics

2.1. Introduction
Canopy microclimate is the climate within and immediately surrounding the vine canopy and is essentially dependent on canopy density, i.e. the amount of foliage and the way that foliage is displayed (Smart and Robinson 1991). Canopy microclimate may be altered by different viticultural techniques that affect the amount and arrangement of leaves, shoots and fruit in space (Smart 1985). Many studies have shown that modifications of canopy microclimate can influence fruit composition either directly or indirectly and hence wine composition and sensory properties of wines (Kliewer 1970, 1977, Smart et al. 1985b, Dokoozlian and Kliewer 1995a, 1995b, 1996, Kliewer and Dokoozlian 2000).

In the present study canopy microclimate was modified by canopy manipulation in order to achieve varying levels of sunlight intensity at the bunch zone. This chapter describes the modifications of the canopy architecture and the assessment of various parameters of canopy microclimate undertaken to determine differences in vine canopy density and to describe the conditions under which the grapes developed and ripened.

2.2. Materials and methods

2.2.1. Location and description of experimental site
The vineyard site selected for this study was at Nuriootpa, in the Barossa Valley district of South Australia, approximately 80 km north-east of Adelaide. The climate of the region is described by Dry and Smart (1988) as warm with mean January temperature (MJT) in the range from 21.0 to 22.9°C and 1817 biologically effective day degrees (Gladstones 1997). Rainfall is moderate (506 mm) with high summer evaporation and low relative humidity. The soil of the site is classified as a light pass fine sandy loam (Northcote 1988).

The vines used in the experiment were Shiraz, clone BVRC 12. The trellis system was a single-wire system, cordon trained and spur pruned. Row and vine spacing were 3.0 m and 2.25 m respectively, with the rows orientated in an east-west direction. Vineyard
management practices were similar to district practices. All treated vines received irrigation of 1 ML/ha from the same main with fixed sprinklers. The irrigation was twice higher than the district average, but this level of irrigation was necessary to produce vigorous shoot growth which could be manipulated to obtain various degrees of sunlight intensity at the bunch zone. There was no Botrytis or powdery mildew infection of the experimental vines.

2.2.2. Viticultural treatments and experimental design
The experiment consisted of three main treatments which altered bunch exposure to sunlight to achieve shaded bunches, moderately exposed bunches and highly exposed bunches. The treatments were as follows:

- **Shaded treatment (ST).** The vine canopies were wrapped in bird nets to constrict the canopy and to create shaded conditions. Bird nets were positioned immediately after fruit set, namely, 14th of December 1999 and 25th of November 2000.

- **Moderately exposed treatment (MET).** No canopy manipulation was undertaken to obtain moderate exposure of bunches to sunlight.

- **Highly exposed treatment (HET).** High posts (2.5m) were placed on the ends of panels with the addition of 3 rows of foliage wires, 50 cm apart. Vine canopies were divided and shoots were trained upwards and downwards. Vertical positioning of shoots, and when required leaf removal around bunches, were carried out periodically during the seasons.

The treatments were arranged in a randomised block design along one row of vines. There were eight replicates of each treatment, each replicate consisting of a panel of 3 vines. The experiment was conducted for two growing seasons, 1999/2000 and 2000/2001.

**Additional treatments**

- **Box treatment (BT).** In the 2000/2001 season an additional treatment (box treatment) was applied on vines of HET. Bunches in a zone of highly exposed bunches were enclosed in boxes designed by Mark Downey, The University of Adelaide. The boxes were made from white polypropylene sheeting (0.6mm) painted black on the inside. They were approximately 250 mm in length and 120
mm deep with the front of the box 150 mm wide and the back 210 mm wide. The boxes were designed to eliminate sunlight (>99.5% of ambient) while allowing air-flow around bunches, without creating any temperature difference between bunches inside the boxes and those in the canopy (Downey pers.comm). Bunches were enclosed in boxes after fruit set, on 24th November 2000.

- **Reference treatment (RT).** The vines from an adjacent row (Shiraz, clone NSW15) that had naturally open canopies of low density and high bunch exposure were used as a reference treatment. The vines received the average district irrigation of 0.5 ML/ha. A decision to include the reference treatment in this study was based on the high quality of RT grapes which was determined in a previous study.

### 2.2.3. Sampling procedure

Samples were collected at regular intervals during berry development starting when berries were pepper-corn size (approximately 3-4 mm in diameter), corresponding to Eichorn and Lorenz (E-L) growth stage 29 (Coombe 1995) and finishing when berries reached a maturity of 26-27 °Brix, corresponding to E-L growth stage 38. In the 1999/2000 season berry sampling commenced on 5th January (45 DAF) and finished on 21st March 2000 (120 DAF). On each sampling date 110 berries were collected in a random manner from the top, bottom, front, back and the middle of bunches from each replicate. Each of the 110-berry samples was then randomly divided into three sub-samples of 50, 30 and 30 berries. In the season 2000/2001 sampling commenced earlier, on 12th of December 2000 (28 DAF) and finished on 4th of March 2001 (110 DAF). On each sampling date three randomly selected bunches from each replicate of ST, MET, HET and RT and one bunch from each replicate of the BT treatment were collected. For each treatment replicate all the berries from bunches were combined and then randomly divided into three sub-samples of 50, 30 and 30 berries.

When berries reached a total soluble solids level of between 23.5 and 24.5 °Brix bunches were sampled for winemaking. Samples of randomly selected 30-bunches and 100-bunches were collected from each treatment replicate in the 1999/2000 and 2000/2001 season respectively. All sample replicates from each treatment were combined and then divided into 3 winemaking replicates. Bunches from MET and HET
were collected on 7th March 2000 while bunches from ST and RT ripened a week later and were collected on 14th March 2000. In the second season, bunches from MFT, HFT, BT and RT were sampled on 5th March, while bunches from ST were sampled on 10th March 2001. Before winemaking three 50-berry samples were randomly sampled from each winemaking replicate.

2.2.4. General sample preparation
The weight of berries of each sub-sample was recorded prior to analysis. The first sub-sample of 50 berries was crushed, centrifuged and juice total soluble solids and pH were measured, using a refractometer and a pH-meter, respectively. Five mL of centrifuged, clear juice was diluted in 25 mL distilled water and kept frozen until analysed for titratable acidity by an automatic titrator. Seeds were separated from flesh and skin and the weight and number of seeds recorded. The other two sub-samples were stored frozen at -20°C until analysed. From the second and third sub-samples seeds were removed under inert gas cover (dry ice) or liquid nitrogen to prevent oxidation. The weight and number of seeds from both sub-samples was recorded. The set of seeds from the second sub-sample was used for determination of physical characteristics: fresh and dry seed weight, seed length and seed coat colour. The set of seeds from the third sub-sample was used for determination of flavan-3-ols and procyanidins (Kennedy and Jones 2001, Peng et al. 2001). The skin and flesh of the second sub-sample was used for determination of total anthocyanins and skin total phenolics (Iland et al. 2000). Skins of the third sub-sample were used for the determination of anthocyanins, flavonols (Peng et al. 2002) and skin tannins (Kennedy and Jones 2001). A detailed description of the extraction procedure and HPLC analysis methods are given in the Materials and Methods section of Chapter 4, pages 58 to 62 and Chapter 5, pages 92 to 94.

2.2.5. Statistical methods
Data were subjected to analysis of variance (AOV) using the general linear model. Separate analysis of variance was carried out for each of the two seasons, and where relevant, at each sampling date. When the AOV indicated that the main effect was significantly different (P<0.05) Fisher’s least significant difference procedure at 95% confidence level was used to discriminate among the means. Correlations between parameters were analysed by simple linear or polynomial regression. P values lower
than 0.05 were considered to be significant. Statistical analyses were performed using Genstat (Version 5, Lawes Agricultural Trust, Rothamsted Experimental Station, UK) and JMP (Version 4.0.4. SAS Institute Inc., Cary, NC). In the chapters that follow, a detailed description of the type of statistical tests used is given where the analyses required differ from those described here.

2.2.6. Vineyard parameters

2.2.6.1. Phenology
The identification of growth stages for the vine was determined using the modified E-L system (Coombe 1995). Flowering occurred in the period between 17th-23rd November in 1999 and from 14th-20th November in 2000. During that period vines went through E-L stages 19-26. Fruit set was estimated as the time when bunches had more than 50% of young enlarging berries, greater than 2 mm in diameter and bunches were at right angles to the stem (E-L stage 27). Fruit set occurred in the period between 23rd-30th November in 1999 and 20th-27th November in 2000. Veraison was defined as the stage when berries softened and began to colour (E-L stages 34-35) and occurred in the period between 18th January and 2nd of February in 2000 and 9th-17th January in 2001. The end of veraison was defined as the time when all berries were coloured.

2.2.6.2. Degree of sunlight intensity at the bunch zone
Sunlight intensity at the bunch zone was determined on a cloudless day between 12 noon and 1 pm using a ceptometer (Decagon Devices, Cambridge, England). Approximately four weeks before harvest readings were made at the fruit zone on both sides of the each vine with the ceptometer positioned parallel to the cordon and pointed upwards. Ambient measures were taken by positioning the ceptometer at the bunch zone height outside the canopy.

2.2.6.3. Shoot and leaf measures

Shoot number and shoot length
The number and length of all shoots per vine were recorded immediately after pruning. An average shoot number and shoot length was then calculated for each treatment replicate.
**Total, main and lateral leaf area**

Leaf area was determined as follows: for each treatment three representative shoots of the approximate length of 0.5, 0.8, 1.0, 1.3, 1.5, 1.8, 2.0 and over 2.3m were collected approximately 3-4 weeks before harvest. For each shoot, the number of leaves on main and lateral shoots was recorded and the mid-rib vein (L1) length measured. Leaf area was calculated by a previously defined regression equation (Equation 1) for Shiraz leaves (P. Dry pers.comm)

\[
\text{Leaf area (cm}^2\text{)} = 0.798 (L1)^2 + 3.63 (L1) \\
\text{Equation 1}
\]

Main leaf area per shoot was calculated by summation of the leaf area of all the leaves on the main shoot. Lateral leaf area per shoot was calculated by summation of the leaf area of all the leaves on the lateral shoots. The relationships between main leaf area and/or lateral leaf area per shoot and shoot length was then determined. Using the appropriate regression equation derived from these relationships and the number and length of shoots per vine (as determined after pruning), the value of the main (MLA) and lateral leaf area (LLA) per vine was calculated. Total leaf area (TLA) per vine was obtained as the sum of the main and lateral leaf area.

**2.2.6.4. Yield**

The total number of bunches was obtained as the sum of the number of bunches on vines prior to harvest and the number of bunches removed for sampling purposes throughout the season. In the first season, the mean bunch weight per replicate was calculated as the average weight of 32 randomly collected bunches at harvest. In the second year the mean bunch weight per replicate was obtained as an average weight of 33 bunches randomly sampled at harvest. Yield per vine was calculated by multiplying the mean bunch weight by the number of bunches per vine.

**2.2.6.5. Pruning weight**

The vines were pruned to 2-bud spurs and the one year old wood from each vine was collected and weighed.
2.2.6.6. Canopy measures

- Canopy surface area was calculated as the sum of the area of the canopy front and back sides (canopy height x length) and the top area (canopy width x length).
- Leaf area/surface area (LA/SA) was calculated as a ratio of total leaf area per surface area.
- Leaf area/fruit weight (LA/FW) was calculated as a ratio of the total leaf area to total fruit weight (yield).
- Yield/pruning weight (Y/PW) was calculated as a ratio of yield and pruning weight.

2.3. Results

2.3.1. Degree of sunlight intensity at the bunch zone
The degree of sunlight intensity at the bunch zone significantly ($P<0.001$) differed between treatments. Measures of light intensity (PAR) at the bunch zone showed that the fruit of ST received less than 5% of available light (<100 PPFD) (Table 2.1). The light intensity at the bunch zone was 10-40% of ambient (300-700 PPFD) for MET and 40-80% of ambient (800-1500 PPFD) for HET (Table 2.1).

2.3.2. Canopy characteristics
Shoot number, shoot length and pruning weight
The number of shoots per vine (60-61.5) and shoots per meter of cordon canopy (25.0-25.6) was not affected by canopy manipulation in the first season while shoot length increased only for HET (Table 2.1). In the following season, canopy shading significantly ($P<0.001$) reduced the number of shoots and shoot length while vertical shoot positioning (HET) increased the number of shoots (Table 2.1).

Pruning weight was not affected by the applied treatments in the first season, while in the second season, pruning weight was greater for MET (4.1kg/vine) than ST (3.6 kg/vine) and HET (3.6 kg/vine), due to lower weight of shoots from HET (48 g) compared to the ST shoots (61 g) or MET shoots (61 g).
Table 2.1. Parameters of sunlight intensity at the bunch zone, canopy growth and canopy size. Measures were taken for the ST, MET and HET treatment in the 1999/2000 and 2000/2001 season. Each value represents the mean of 8 replicates. Means in columns followed by different letters are significantly different within one season.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ST</td>
<td>MET</td>
</tr>
<tr>
<td>% PAR at the bunch zone</td>
<td>2.4  a</td>
<td>30.8 b</td>
</tr>
<tr>
<td>PPFD at the bunch zone</td>
<td>43 a</td>
<td>564 b</td>
</tr>
<tr>
<td>Main LA/vine (m³)</td>
<td>10.5 a</td>
<td>11.4 b</td>
</tr>
<tr>
<td>Lateral LA/vine (m³)</td>
<td>2.2 b</td>
<td>0.8 a</td>
</tr>
<tr>
<td>Total LA/vine (m³)</td>
<td>12.7 ab</td>
<td>12.2 a</td>
</tr>
<tr>
<td>% of laterals</td>
<td>17.2 b</td>
<td>6.9 a</td>
</tr>
<tr>
<td>Shoot no/vine</td>
<td>61.4</td>
<td>60.1</td>
</tr>
<tr>
<td>Shoot no/m canopy</td>
<td>25.6</td>
<td>25.1</td>
</tr>
<tr>
<td>Shoot length (m)</td>
<td>1.0 a</td>
<td>1.1 ab</td>
</tr>
<tr>
<td>Canopy volume (m³)</td>
<td>3.2 a</td>
<td>5.0 b</td>
</tr>
<tr>
<td>Ratio height: width</td>
<td>0.8 a</td>
<td>0.9 b</td>
</tr>
<tr>
<td>Surface area (m²)</td>
<td>8.0 a</td>
<td>10.2 b</td>
</tr>
<tr>
<td>Pruning weight (kg)</td>
<td>2.8</td>
<td>2.8</td>
</tr>
<tr>
<td>LA/SA</td>
<td>1.6 b</td>
<td>1.2 a</td>
</tr>
</tbody>
</table>

ns not significant, ***, *** indicate significance at P<0.05, P<0.01, P<0.001 respectively
Leaf area
The effect of canopy manipulation on total leaf area was more prominent in the second than in the first season (Table 2.1). The canopy shading reduced both MLA and LLA which resulted in 30% decrease of TLA for ST, compared to MET and HET. MLA (15.4 m² and 15.0 m²) and TLA (20.8 m² and 22.2 m²) was similar for MET and HET respectively, although growth of LLA was more intensive for HET (7.1 m²) than MET (5.4 m²) (Table 2.1).

Canopy architecture
The ratio of canopy height to canopy width was significantly (P<0.001) greater for HET (1.3 and 1.4) than MET (0.9 and 1.0) and ST (0.8 and 0.9) (in the first and second season respectively) (Table 2.1). Similarly surface area (SA) was significantly (P<0.001) higher for HET (10.7 and 12.0) than MET (10.2 and 10.5) and ST (8.0 and 9.5).

2.3.3. Yield and vine balance
The mean bunch weight was similar for all treatments within the same season (69-72 g and 133-146g in the first and second season respectively), while the number of bunches per vine and per shoot varied between treatments and seasons (Table 2.2). Yield per vine was similar for all treatments except for ST in the first season (Table 2.2). There was no significant difference in Y/PV between treatments in both seasons while LA/FW was lower for ST and MET in the first season and for ST in the second season (Table 2.2).

There were significant differences (P<0.001) in yield, LA/FW and Y/PW between seasons. Yield (kg/vine) was five fold greater in the second than in the first season which was due to an increase in both bunch number and bunch weight (Table 2.2). Thus, the ratio leaf area to fruit weight (LA/FW) was higher in the first season (17.6, 17.8 and 20) than in the second season (10.4, 15.6 and 15.5) for ST, MET and HET respectively) and the ratio yield to pruning weight (Y/PW) was lower in the first season (1.0–1.6) than in the second season (4.5-5.0).
Table 2.2. The average number of bunches per vine and per shoot, average bunch weight and yield per vine. Bunches of the ST, MET and HET treatment were sampled in the 1999/2000 and 2000/2001 season. Each value represents the mean of 8 replicates. Means in columns followed by different letters are significantly different within one season.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Year</th>
<th>Bunch no/vine</th>
<th>Bunch no/shoot</th>
<th>Bunch weight (g)</th>
<th>Yield/vine (kg)</th>
<th>Y/PW</th>
<th>LA/FW</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST</td>
<td>2000</td>
<td>61.4 c</td>
<td>1.01 b</td>
<td>72.3</td>
<td>4.42 b</td>
<td>1.6</td>
<td>17.6 a</td>
</tr>
<tr>
<td>MET</td>
<td>2000</td>
<td>42.0 ab</td>
<td>0.72 a</td>
<td>69.2</td>
<td>2.91 a</td>
<td>1.1</td>
<td>17.7 a</td>
</tr>
<tr>
<td>HET</td>
<td>2000</td>
<td>39.1 a</td>
<td>0.65 a</td>
<td>69.0</td>
<td>2.71 a</td>
<td>1.1</td>
<td>20.1 b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>***</td>
<td>***</td>
<td>ns</td>
<td>***</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>ST</td>
<td>2001</td>
<td>122.5 b</td>
<td>2.09 b</td>
<td>141.3</td>
<td>17.35</td>
<td>5.1</td>
<td>10.4 a</td>
</tr>
<tr>
<td>MET</td>
<td>2001</td>
<td>134.9 c</td>
<td>2.06 b</td>
<td>133.2</td>
<td>17.96</td>
<td>4.5</td>
<td>15.8 b</td>
</tr>
<tr>
<td>HET</td>
<td>2001</td>
<td>112.2 a</td>
<td>1.49 a</td>
<td>145.6</td>
<td>16.32</td>
<td>4.6</td>
<td>15.5 b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>***</td>
<td>***</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

ns not significant, **,**,**,** indicate significance at P<0.05, P<0.01, P<0.001 respectively

2.4. Discussion

*Canopy manipulation and the level of sunlight intensity at the bunch zone*

The canopy manipulations affected the arrangement of leaves and shoots within the canopy and hence, the canopy microclimate. Both surface area and the ratio canopy height to width were positively correlated with PAR indicating that the modifications of vine architecture were an effective means to create differences in the level of sunlight intensity at the bunch zone. Vertical shoot positioning for HET improved sunlight interception at the bunch zone to 40-80% of ambient (800-1500 PPFD) compared to the level of 10-40% of ambient at the bunch zone (300-700 PPFD) provided by natural positioning of shoots for the MET treatment. Shaded conditions created for the ST treatment reduced sunlight intensity to less than 5% of ambient (<100 PPFD). These levels of sunlight intensity at the bunch zone provided different conditions for fruit development and ripening which would likely result in differences in fruit and wine composition (Smart et al. 1985b, Intrieri 1987, Morrison and Noble 1990, Macaulay and Morris 1993, Hunter 1998, Petrie et al. 2000b).
The vine response to canopy manipulation

The vine is believed to have phenotypic adaptation to low light intensity through modifications in the vine growth habit and/or leaf anatomy (Schultz 1993, Schultz et al. 1996). By shading the ST treatment, it was likely that there were a high percentage of shaded leaves with limited photosynthetic capacity. The vine response to canopy shading in the second season was reflected in the reduced number of shoots and the foliage area by 30%. This indicated that the vines of the ST treatment showed a tendency to naturally create more open canopies which would increase both direct and diffuse light. At the same time, the increased proportion of lateral leaf area, observed in both seasons, may be a result of the vine’s inclination to fill the gap frequency which would compensate the limited photosynthetic capacity. These results support findings that have shown that the response of the vine to shading depends on the level of PAR available during leaf development and shaded vines adapt by modifying a growth habit which would enable the production of certain amount of photo-assimilates (Smart et al. 1982b, Smart et al. 1985b, Schultz 1995).

The vine response to the vertical positioning of shoots upwards and downwards (HET) resulted in increased growth of lateral leaf area and an increased number of shoots (Table 2.1). This could be a result of temperature and sunlight intensity on vegetative growth as it was shown that lateral shoot growth of Shiraz vines increased with increasing temperature over the whole range between 15 and 35 °C (Buttrose 1969). High light intensity was also more favourable for lateral shoot growth relative to main growth for other varieties such as Muscat Gordo Blanco and Ohanez (Buttrose 1968, 1969). Furthermore, the increase of lateral growth as a result of vertical shoot positioning or by dividing the canopy was observed in vines of Cabernet Sauvignon, Merlot and Sultana (May et al. 1973, Mabrouk et al. 1997). In the present study, by vertical shoot positioning, the leaf area exposed to high level of sunlight intensity increased and it was likely that a greater foliage area was exposed to higher than optimal temperature (30 °C) for leaf photosynthesis (Iland 1989a). Other studies reported strong interactions between sunlight intensity and leaf temperature, water status and humidity which affected photosynthesis (Kriedemann 1968, Smart et al. 1982a, During and Harst 1996). However, differences in sunlight interception would have effects on both leaf photosynthetic activity and on berry metabolism and it is not possible to separate these effects in relation to the differences in berry composition.
In the first season a greater yield was observed only for the ST treatment due to higher number of bunches than the other treatments (Table 2.2). There was a significant difference ($P<0.001$) between seasons with both higher number of bunches and mean bunch weight which resulted in 4 times greater yield in the second than in the first season (Table 2.2).

*Canopy manipulation and vine balance*

Gladstones (1992) defined that the vine is in balance “when vegetative vigour and fruiting load are in equilibrium and consistent with high fruit quality”. Various indicators for assessing the balance between vegetative and fruit growth have been used and optimal values have been proposed in order to create optimal conditions in vine canopies for the production of high quality fruit. Several studies have shown that a particular ratio of leaf area to fruit weight (LA/FW) (5-10 cm$^2$/g) is required for adequate maturation of fruit (Kliewer and Weaver 1971, Smart and Robinson 1991, Petrie et al. 2000a, 2000b). The results showed that the ratio LA/FW was closer to the optimal value of 5-10 cm$^2$/g in the second season (10-15 cm$^2$/g) than in the first season (17-20 cm$^2$/g) when excessive shoot and foliage growth occurred along with a low yield.

An optimal fruit weight to pruning weight ratio of 5-10 kg/kg has been widely used as a criterion of vine balance (Bravdo et al. 1984, Smart 1985, Smart and Robinson 1991, Kliewer and Dookozlian 2000). In the present study, despite the differences in the canopy growth and architecture, there was no significant difference in the ratio FW/PW between treatments within the same season, but the value of the ratio was closer to the optimal range in the second season, than in the first one, due to the low yield in the first season. The results from this study indicated that the whole vine performance was improved in the second season which resulted in higher vegetative and fruit growth and greater vine balance than in the first season. These differences along with seasonal climatic differences can help explain the disparity in berry and wine composition between seasons.
2.5. Conclusion

- The applied treatments were successful in creating differences in sunlight intensity at the bunch zone. Sunlight intensity at the bunch zone of the ST treatment was reduced to less than 5% of ambient (<100 PPFD). Vertical shoot positioning for HET increased sunlight interception at the bunch zone to 40-80% of ambient (800-1500 PPFD) compared to the level of 10-40% of ambient at the bunch zone (300-700 PPFD) provided by natural positioning of shoots for the MET treatment. Within each season, the differences in sunlight intensity at the bunch zone are likely to play a large role in creating differences in berry and wine composition.

- The indicators of vine balance (LA/FW and Y/PW) have shown that the vine performance was more affected by seasons than by applied treatments. The average bunch weight and yield per vine was similar for all treatments within one season, but significantly different between seasons.
Chapter 3 – Developmental changes in morphological characteristics of seeds and their relationships to berry development

3.1. Introduction
Seed and berry growth and development occur simultaneously and the three phases of berry growth are marked not only by changes in the diameter, length and volume of berries, but also by changes in the weight of seeds (Harris et al. 1968, Coombe 1973, Alleweldt et al. 1984). It is likely that the phases of berry development are also linked to other morphological and chemical changes in seeds. The studies reported in this chapter investigate a range of morphological changes in seeds and their relationship to berry development. A number of studies have shown that different light conditions at the bunch zone affect berry development and berry composition (Crippen and Morrison 1986a, 1986b, Rojas-Lara and Morrison 1989, Morrison and Noble 1990, Gao and Cahoon 1994) but there has been little research on the effects of sunlight intensity at the bunch zone on seed development.

3.2. Materials and methods
The set of seeds from one 30-berry sub-sample (as previously described in section 2.2.4.) was used for the determination of physical characteristics of seeds: fresh and dry weight, seed length and seed coat colour. Twenty seeds from each replicate were randomly selected, fresh seed weight and length measured and then seeds were dried over 24 hours in an oven at 60 °C and dry seed weight and length of dry seeds measured. Water loss was calculated as a difference between fresh and dry seed weight. A randomly selected sample of 20 seeds from each replicate at each sampling date was used for the description of the seed appearance during seed development. The value of seed coat colour was determined using a seed colour chart (Appendix 1). The seed colour chart comprised 12 colours with corresponding numbers, starting with a bright green, through green-yellow (colours 1-4) to yellow (colour 5) and then through yellow-brown (colours 6-9) to dark brown colours (colours 10-12). For each seed, the top and bottom sides of the seed were separately matched to one colour. An overall seed colour value was calculated by averaging the values obtained for tops and bottoms. Detailed calculation of the seed coat colour is given in Appendix 1.
3.3. Results

3.3.1. Developmental changes in the morphology of the grape seed
A detailed description of changes in the external appearances of the seeds and their features (beak, chalaza and raphe) during seed development is given in Figure 3.1. At the beginning of seed development (approximately 20-30 days after flowering (DAF)) seeds were very small, bright green, soft and susceptible to damage during separation from berry flesh. In the period from 30-60 DAF (until veraison) seeds remained soft, pliable and with smooth texture of all surfaces. The chalaza was situated in the centre of the dorsal side, but close to veraison, slight ruminations of the fossettes caused displacement of the chalaza towards the notch and seeds enlarged at their basal end. Approximately 60 DAF seeds reached their full shape and size, with a fully developed beak. Thereafter, seed surfaces lost their smoothness and softness, becoming hard and rough in texture and seeds appeared progressively more brown. 92 DAF seeds appeared to be fully formed with a straight, slightly rough and dark coloured beak. The chalaza was positioned toward the notch, wrinkled, slightly sunken and dark in colour. The raphe was distinctly noticeable on both sides of the seed. Fossettes were dark in colour and hard in texture. In the following days (106-120 DAF) seeds continued to change colour becoming more uniformly dark brown.
Seeds were very small, bright green, soft and pliable.

Seeds increased in size and were green-yellow and soft. The chalaza appeared as a slight light circle in the middle of the dorsal side.

Seeds appeared to be soft and green, pliable and with smooth texture on all surfaces. They reached 90% of their full length. The chalaza was situated in the centre of the dorsal side of the seed as a light yellow smooth circle. On the ventral side of the seed, a longitudinal ridge (keel) appeared as a light coloured narrow line. There were no signs of rumination of the fossettes.

Seeds were still soft, with smooth surfaces and enlarged on their basal end. Slight ruminations of the fossettes appeared as two small dark regions at both sides of the raphe. The chalaza was just noticeable as a circle of the same colour as fossettes.

Seeds reached their full shape and size, with a fully developed beak. They were dark green, smooth on the both surfaces, and harder in texture. The chalaza was now smooth, dark in colour and situated more towards the notch. The rumination of fossettes continued and the raphe was very distinct and sharp.

Seeds changed colour from yellow to yellow-brown, rumination of fossettes was distinct and the chalaza started to sink and wrinkle.

Seeds became progressively more brown on the dorsal side. Changes on the dorsal side were more distinct than those on the ventral side. The light brown chalaza was now more sunken and wrinkled. Fossettes continued to ruminate. Seed surfaces lost their smoothness and softness, becoming harder and rougher in texture.

Seeds became more brown on the ventral side. Ruminations of fossettes and wrinkling of the chalaza continued.

Rumination of fossettes was more noticeable, the chalaza more sunken and wrinkled. The beak started to wrinkle and become darker in colour. The raphe was prominent, but still slightly lighter in colour.

Seeds were fully formed with a straight and slightly rough, dark coloured beak. The chalaza was positioned toward the notch, wrinkled, slightly sunken and dark in colour. The raphe was distinctly noticeable. Fossettes were dark in colour and hard in texture. Thereafter, seeds continued to darken in colour.

**Figure 3.1.** Linear diagram of developmental changes in the appearances of Shiraz grape seeds.
Colour of the grape seed coat

Colour of the grape seed coat changed from bright green (colour 1), yellow (colour 5), through light brown (colours 6-9) to dark brown (colours 10 and 11) (Figure 3.2). At veraison seeds from the ST treatment had lower colour values (5.8 and 5.0) compared to MET (6.3 and 5.4) and HET (6.2 and 5.2) in the first and second season respectively. This was probably due to higher proportion of green than coloured berries in the sample for the ST treatment. Later in development when berries reached the stage of maturity of 25-26 °Brix, the value of seed coat colour was similar for all treatments within the same season (10.7, 10.7, 10.8 and 10.1, 10.2 and 10.0 for ST, MET and HET in the first and second season respectively).

![Figure 3.2](image_url)

Figure 3.2. Changes in seed coat colour values during berry development and ripening under different light conditions. The berries of the following treatments: • ST (1-10% sunlight at the bunch zone), ■ MET (10-50% sunlight at the bunch zone) and ▲ HET (40-80% sunlight at the bunch zone) were sampled during (a) 1999/2000 and (b) 2000/2001 season. Each point represents the mean of eight replicates and error bars represent ± standard error of the mean.

3.3.2. Developmental changes in berry weight

Berry sampling commenced at 45 DAF (1999/2000 season) and 28 days after flowering (DAF) (2000/2001 season) and covered several developmental stages between fruit setting and post-harvest. The stages, as described by Coombe (1995), represent E-L stages 29, 31, 32, 33, 35, 36, 37, 38 and 39 of berry development (E-L stages 29-33) and berry ripening (E-L stages 33-39). Veraison (E-L stage 35), defined by berry softening and berry colouring, occurred between 64-73 DAF (1999/2000 season) and
64-70 DAF (2000/2001 season). Berries were harvested for winemaking when the concentration of total soluble solids was approximately 24 °Brix (E-L stage 38). Some fruit was retained on the vine so that developmental changes could be monitored post-harvest.

The pattern of berry growth
The pattern of berry growth followed a typical double sigmoid pattern except when berry shrinkage occurred after 91-92 DAF (Figure 3.3). Initially berries were green, hard and small (0.34, 0.29 and 0.30 g for ST, MET and HET respectively at 28 DAF in the second season). During the lag phase (58-64 DAF or 49-56 DAF in the first and second season respectively) the gain in berry weight was very slow and berries gained only 0.03-0.06 g. After the lag phase, growth was rapid until berries gained maximal weight at approximately 91-92 DAF (Figure 3.3). In the first season, maximal berry weight was reached at around 91 DAF and it was as follows: 1.54, 1.50 and 1.62 g for ST, MET and HET respectively and thereafter decreased to 1.40, 1.33 and 1.46 g for ST, MET and HET respectively (120 DAF). In the second season, around 92 DAF, berry weight reached its maximum of 1.23, 1.17 and 1.30 g (ST, MET and HET respectively) and thereafter decreased to 1.12 (117 DAF), 1.06 and 1.10 g (110 DAF) for ST, MET and HET respectively.

Differences in berry weight between treatments and seasons
In the early stages of berry development there was no difference in berry weight between treatments. The significantly (P<0.001) lower berry weight of ST (8-15% lower than HET and 6-10% lower than MET) was observed only during veraison in the first season, but neither at the later stages of berry development and ripening or in the second season. However, after veraison, berry weight of ST was greater than berry weight of MET, although the difference was significant (P<0.001) only at 79 and 98 DAF in the second season. Comparison of the growth curves for MET and HET showed a significant difference (P<0.001) only in the period between 92 and 120 DAF in the first season, and at only one sampling date (79 DAF) in the second season (berry weight of HET was 8-10% greater than MET), but afterwards (110-113 DAF) the difference (3-5%) was not statistically significant (P<0.05) (Figure 3.3). There was a significant difference (P<0.001) in berry weight between seasons with berry weight being 20% greater in the first than the second season.
3.3. Developmental changes in seed weight
Seed samples were collected at regular intervals between 48 and 120 DAF and 28-113 DAF (in the first and second season respectively); these intervals covered several seed developmental stages. At the beginning of berry development, the contribution of the seed weight to the berry weight was around 7%, but this proportion rapidly decreased to a plateau of around 2.5% when berries reached their maximal weight.

Developmental changes in fresh seed weight
The pattern of changes in fresh seed weight apparently followed a biphasic curve (Figure 3.4). Until approximately 56 DAF seeds gained more than 95% of their maximal weight. The maximal fresh seed weight was reached at around 64 DAF and was as follows: 41.9, 42.9, 43.3 mg and 28.7, 29.7 and 29.7 mg (ST, MET and HET in the first and second season respectively). Thereafter the weight of fresh seeds declined by 20% until 91 DAF and then 5% more until the end of the sampling period when the mean fresh seed weight was: 33.4, 32.5, 33.4 mg (120 DAF) and 24.3, 23.3 and 23.7 mg (100 DAF) (ST, MET and HET in the first and second season respectively). This would suggest that the trend of developmental changes in fresh seed weight may be divided
into two phases: i) a phase of a rapid increase until the maxima is reached (approximately 64 DAF) and ii) a period of a decrease until a plateau is reached (approximately 91 DAF).

Developmental changes in dry seed weight

The pattern of developmental changes in weight of dry seed followed a biphasic curve (Figure 3.4). It appears that initially the gain in dry seed weight was rapid and by the end of veraison (73 and 64 DAF in the first and second season respectively) seeds gained more than 95% of their maximal dry seeds weight (Figure 3.4). After veraison, until approximately 91 DAF, increase in dry seed weight continued at a slower rate. In both seasons, maximal dry seed weight was observed for all treatments at around 91-92 DAF and it was as follows: 25.4, 25.6, 25.4 mg and 18.1, 17.5 and 17.8 mg (ST, MET and HET in the first and second season respectively). After 91-92 DAF there was no significant difference in dry seed weight between different sampling dates in both seasons, although it should be noted that in the 1999/2000 season, at 113 DAF, dry seed weight surprisingly increased, following the increase in fresh seed weight and berry weight. The contribution of dry seeds weight to total seed weight increased from 50% (at the time of maximal fresh weight) to 80% (at the time of maximal dry weight). The results would suggest that the trend of changes in dry seed weight was increasing until the maximum was reached (approximately 91-92 DAF) and thereafter plateaued (Figure 3.4).

Developmental changes in difference between fresh and dry seed weight and seed length

The pattern of the difference between fresh and dry seed weight (water loss) showed a declining trend until 91-92 DAF and thereafter remained at a plateau of 6-9 mg and 5-6 mg (in the first and second season respectively) (Figure 3.4).

Maximal length of seeds of 5.3, 5.3, 5.4 mm and 5.1, 5.0, 5.0 mm (ST, MET and HET in the first and second season respectively) was reached towards the end of veraison (64-73 DAF) and thereafter remained constant.
Figure 3.4. Changes in weight of fresh seeds, dry seeds and water loss (difference between fresh and dry seed weight) during berry development and ripening under different light conditions. The berries of the following treatments: ♦ ST (1-10% sunlight at the bunch zone), ■ MET (10-50% sunlight at the bunch zone) and △ HET (40-80% sunlight at the bunch zone) were sampled during (a) 1999/2000 and (b) 2000/2001 season. Each point represents the mean of eight replicates and error bars represent ± standard error of the mean.

**Differences in seed weight between treatments and seasons**

A significant difference ($P<0.001$) in the mean weight of fresh and dry seeds and seed moisture (difference between fresh and dry seed weight) between treatments during the period of berry and seed development was observed at some sampling dates, but there was no consistent pattern which would indicate any treatment effect on fresh and dry seed weight.
The number of seeds per berry was significantly ($P<0.001$) less in the first season (1.75) than the second one (2.50). There were significant differences ($P<0.001$) in fresh seed weight, dry seed weight, difference between fresh and dry seed weight and seed length between seasons. The mean weight of fresh and dry seeds and difference between fresh and dry seed weight was 30% greater and seed length 5-8% greater in the first than the second season.

### 3.3.4. Relationships between developmental changes in berry weight and seed weight

A curvilinear relationship was established between berry and seed weight indicated that berry and seed development were closely related (the coefficient of determination ($r^2$) was 0.57 and 0.47 ($P<0.001$) in the first and second season respectively) (Figure 3.5.a). The fitted polynomial (top) curve for fresh seed weight showing a maximum at veraison and a minimum at approximately 92 DAF which coincided with maximal berry weight. The trend indicated that the events in the course of berry development were related to developmental changes in fresh seed weight. When divided into three periods (pre-veraison, veraison and post-veraison) (Figure 3.5.b) the relationship between berry and fresh seed weight was as follows: i) until veraison (64 DAF) the relationship was linear ($r^2=0.12$ (ns) and 0.54 ($P<0.001$) for the first and second season respectively) which indicated a positive inter-relation between seed and berry development, ii) during veraison there was only a weak linear relationship in the second season ($r^2=0.15$), and iii) in the post-veraison period the fitted polynomial curve indicated an inverse highly correlated relationship ($r^2=0.52$ ($P<0.001$) and 0.35 ($P<0.01$) for the first and second season respectively) between berry weight and fresh seed weight. All three curves crossed at the point that corresponded to a berry weight of 0.85 g and 0.66 g which occurred in the period of 64-73 DAF and 56-64 DAF (in the first and second season respectively) (Figure 3.5.b). This timing corresponded to the period immediately after the end of lag phase, indicating a relationship between the completion of seed growth (measured as fresh seed weight) and the beginning of berry colouring.
The relationship between dry seed weight and berry weight during the whole period of berry development and ripening was best fitted by a polynomial curve of the second degree (Figure 3.5.a). The coefficient of determination \( r^2 \) of 0.66 and 0.81 in the first and second season respectively indicated highly significant correlation between variables \( (P<0.001) \). The fitted polynomial (bottom) curve for dry seed weight plateaued as berry weight approached its maxima (Figure 3.5.a). When the course of berry development and ripening was divided into three periods (pre-veraison, veraison and post-veraison) (Figure 3.5.b) the relationship between berry weight and dry seed weight was as follows: i) until veraison the relationship was linear \( (r^2=0.56 \ (P<0.001) \) and 0.73 \( (P<0.001) \) for the first and second season respectively), ii) during veraison the relationship remained linear and while still significant, the correlation weakened \( (r=0.30 \ (P<0.05) \) and 0.44 \( (P<0.01) \) for the first and second season respectively) which was probably due to the slow increase in dry seed weight and berry weight and iii) in the post-veraison period the fitted curve deviated from linearity which lowered the correlation \( (r^2=0.33 \ (P<0.01) \) and 0.05 (ns) for the first and second season respectively). In each season all three curves crossed at one point that corresponded to berry weight of 0.85 g and 0.70 g which occurred in the period of 64-73 DAF in both seasons (Figure 3.5.b). This would suggest that gross accumulation of dry seed weight was closely related to the early stages of berry development. As berry weight approached its maximum, the rate of increase in dry seed weight was slower than the rate of the
increase in berry weight which probably contributed to the weaker relationship between variables. However, both dry seed weight and berry weight reached their maxima at the same time (approximately 91-92 DAF).

Figure 3.5.b. Relationship between berry weight and fresh and dry seed weight during berry development and ripening in (a) 1999/2000 and (b) 2000/2001 season. Different periods of berry development and ripening are marked by colour of the seed coat as follows: ■ (green) represents the period pre-veraison, ▲ (yellow) represents veraison and ● (brown) represents post-veraison. Polynomial curves are fitted for fresh and dry seed weight during each period in both seasons using the data from all treatments.

All the above data resulted from samples that were randomly collected from the whole population of berries at each particular date. Samples collected in the period of veraison contained proportions of green and red coloured berries. For the samples collected during the veraison period in 2000/2001 season, additional measurements of seed weight and berry weight were undertaken on berries that were separated on the basis of green and red coloured berries (Table 3.1).
Table 3.1. Berry weight, fresh and dry seed weight and seed dimensions of seeds from green and red coloured berries at veraison (64 DAF). Each value represents the mean of 30 berries of 24 replicates. Means in columns (± standard error of the mean) followed by different letters are significantly different.

<table>
<thead>
<tr>
<th>Berry colour</th>
<th>Berry weight (g)</th>
<th>Fresh seed weight (mg)</th>
<th>Dry seed weight (mg)</th>
<th>Difference: fresh and dry (mg)</th>
<th>Seed length (mm)</th>
<th>Seed width (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green</td>
<td>0.7±0.0</td>
<td>30.6±0.3</td>
<td>17.8±0.2</td>
<td>13.5±0.1</td>
<td>4.8±0.4</td>
<td>3.2±0.2</td>
</tr>
<tr>
<td>Coloured</td>
<td>0.8±0.0</td>
<td>32.9±0.3</td>
<td>19.5±0.3</td>
<td>13.2±0.2</td>
<td>4.8±0.4</td>
<td>3.3±0.3</td>
</tr>
</tbody>
</table>

*** ns not significant, ***, **** indicate significance at P<0.05, P<0.01, P<0.001 respectively

Berry weight of red coloured berries was significantly higher compared to green berries. Fresh seed weigh and dry seed weight were significantly higher in seeds from red coloured berries than seeds from green berries (Table 3.1). There was no significant difference in seed length while the difference in seed width was significant at P<0.05. These data would support the previous indications that berries start colouring when seed growth is complete as seeds did not continue to enlarge at next measurement date.

3.4. Discussion

The objective of Chapter 3 was to identify developmental stages in the course of seed growth and to examine the relationships between seed and berry growth and development. The hypothesis was that sequences of seed growth coincide with sequences of berry growth and ripening.

3.4.1. Seed growth pattern

The growth pattern of grape seeds has not been studied in such complexity as has been done for seeds of other species, in particular cereals seeds. In general the growth pattern of cereal seeds have been divided into the following phases: i) phase of histodifferentation, ii) phase of expansion and iii) phase of maturation and drying (Bewley and Black 1994). These phases are clearly separated by changes in seed fresh weight, dry weight and water content. Similar stages in the growth pattern of grape seeds were indicated in previous studies which focused primarily on berry development in different grape varieties. In a morphological study of Delaware grapes (Nakagawa
and Nanjo 1965) reported a rapid increase in seed size and fresh seed weight during the first cycle in berry development after which it plateaued. Staudt et al. (1986) proposed a biphasic curve pattern of seed growth which was based on the changes in dry seed weight for the grape variety Bacchus. However, these studies have not examined other relationships between fresh seed weight, dry seed weight and difference between fresh and dry seed weight in relation to seed growth and development.

In this study, the analysis of developmental changes of Shiraz seeds, which included changes in fresh seed weight, dry seed weight and difference between fresh and dry seed weight, as well as the changes in physical appearances of seeds (seed morphology) indicated three phases in seed growth and development (Figure 3.6). During the first phase seeds rapidly gained fresh weight and increased in size. This would be associated with cell division and differentiation (Pratt 1971). By the end of this phase, fresh seed weight reached its maximum and dry seed weight gained more than 90% of its maximum. Colour of the seed coat changed from bright green to green-yellow. The second phase was relatively short and was marked by a slow increase in dry seed weight and a decline in water content, while fresh seed weight remained stable. During this phase the basal end of the seed enlarged considerably, causing displacement of the chalaza towards the notch. This event could be associated with cell expansion to accumulate reserve materials (Pratt 1971). During the second phase the seed coat had a maize yellow colour. The third phase was determined by maximal gain in dry weight and a rapid decrease in fresh weight and water content. Rumination of fossettes, roughness and hardening of seed surfaces and the changes in seed colour from light brown to dark brown were distinct manifestations of a process of seed drying and maturation.

Based on these events, grape seed growth and development was divided into three phases as follows: 1) a phase of seed growth, 2) an intermediate phase and 3) a phase of seed drying and maturation (Figure 3.6). The proposed growth pattern of the grape seed was similar to the growth pattern of cereal seeds although the second phase in the grape seed growth was shorter and not as clearly defined by the changes in fresh and dry seed weight as it was in cereal seeds growth. The pattern of grape seeds growth showed not only relations between fresh seed weight, dry seed weight and water content, but also relations with seed coat colour and formation of seed features (seed morphology).
Figure 3.6. Notional diagram of the phases in the course of seed growth showing developmental changes in seed coat colour, formation of the seed features and changes in fresh seed weight, dry seed weight and water loss during berry development and ripening for the following treatments: • ST (1-10% sunlight at the bunch zone), • MET (10-50% sunlight at the bunch zone) and Δ HET (40-80% sunlight at the bunch zone). Each point represents the mean of eight replicates and error bars represent ± standard error of the mean. Curves are fitted for fresh weight, dry weight and water loss in (−) 1999/2000 and (---) 2000/2001 season.
3.4.2. Relationship between berry and seed development

The relationship between seed weight and berry weight in both seasons was defined as a highly correlated, polynomial relationship. The significant difference in seed number per berry, seed weight and berry weight between seasons had no or little effect on the relationship between seed and berry weight. This supports the findings of other studies which reported that the number of seeds and the total weight of seeds per berry are highly correlated to final berry size, berry fresh and dry weight (Ebadi et al. 1996).

Various studies have indicated that seed and berry growth and development occur simultaneously (Coombe and Hale 1973), thus the objective of this study was to closely examine the relationship between each phase in seed development with the phases in berry development. The growth of a grape berry follows a double sigmoidal curve pattern and consists of two phases of rapid growth separated by a phase of slow growth or lag phase (Figure 3.3). During the first cycle of berry growth and development (0-60 DAF) fresh seed weight increased rapidly reaching its maximum at the end of the lag phase of berry growth. Seeds also increased in size until they reached the maximum length and width after which berry colouring started. The positive correlation between berry weight and seed weight supported the finding that in the first cycle of rapid berry growth the rate of pericarp cell division was positively correlated with the growth rate of developing seeds (Coombe 1960, Coombe and McCarthy 2000). Ojeda et al. (1999) suggested that seed growth could have a positive effect on cell mitosis rather than on cell enlargement. A significant difference (P<0.001) in seed dimensions (fresh and dry seed weight and seed length) between seeds from green and coloured berries sampled at the same date confirmed the association between seed growth and the beginning of berry colouring and this study provided further evidence that the beginning of veraison and berry colouring may be associated with cessation of seed growth.

In the second cycle of berry growth, which started with veraison, fresh seed weight and water content declined while dry seed weight steadily increased until the maximum was reached (at approximately 92 DAF), which coincided with maximum berry weight. Other studies of grape berry development have also shown that the maximum berry weight coincided with maximal dry seed weight and maximal embryo length (Nakagawa and Nanjo 1965, Staudt et al. 1986, Ebadi et al. 1996). When maximal dry seed weight was reached, the degree of difference between fresh and dry seed weight
plateaued and formation of the beak, chalaza and raphe were accomplished. Thereafter, berries started to shrink; a finding that supports the observation of the growth pattern for Shiraz berries by McCarthy (1999), McCarthy and Coombe (1999) where berry weight decline commenced at approximately 91 DAF.

The findings of this study provided further information on seed morphology, seed and berry development and the relationships between them. The data clearly indicated that each sequence of seed development coincided with the sequence in berry development.

3.4.3. Influence of sunlight intensity at the bunch zone on the seed number, seed weight and berry weight
It appears that sunlight intensity at the bunch zone had only a small effect on fresh and dry seed weight or the number of seeds per berry within the same season. The differences in berry weight due to the effect of sunlight intensity at the bunch zone also showed no steady pattern. In general, the mean berry weight of berries that were grown under moderate light intensity (10-40% of ambient) (MET) was slightly lower while the mean berry weight of berries grown under high light intensity (40-80% of ambient) (HET) was slightly higher than the other treatments. Greater weight of berries grown under reduced light conditions (<5% of ambient) (ST) may be related to the difference in temperature at the bunch zone. Other studies have shown that as a result of lower berry temperature, shaded berries had lower transpiration rate and higher turgor pressure with consequent enlargement in berry size (Reynolds et al. 1986, Blanke and Leyhe 1987). Similar results were reported for Cabernet Sauvignon (Crippen and Morrison 1986a), Serval Blanc (Reynolds et al. 1986), Pinot noir (Price et al. 1995a) and Muscat of Frontignan (Bureau et al. 2000). Increased berry weight with higher sun exposure (HET) was probably due to increased photosynthesis and carbohydrate transport as a greater proportion of leaves of HET were exposed to high sunlight intensity. Koblet (1975) indicated that leaves shaded with other leaves showed practically no export of carbohydrate. Dookozlian and Kliwer (1996) reported that bunch shading affected berry weight and berries of Pinot Noir and Cabernet Sauvignon that were grown without light during the early stages of berry development (stage I and II and during stage I, II and III) had lower berry weight than the control treatment while elimination of light only in stage III had no effect on berry weight. The authors
suggested that this effect may be due to light-mediated effects on cell division and/or cell enlargement, particularly during the initial stage of growth.

However, it appears that the seed number, seed fresh and dry weight and berry weight were more affected by season (20% and 30% greater in the first season than in the second season for berry and seed weight respectively) than by sunlight intensity at the bunch zone.

3.5. Conclusion

- Three phases in grape seed growth were defined as follows: i) a phase of seed growth, ii) an intermediate phase and iii) a phase of seed drying and maturation. These phases were based on developmental changes in fresh seed weight, dry seed weight, water content, seed width and length and seed physical appearances that include formation of seed features and seed coat colour.

- During the development period the following sequences of seed development coincided with sequences of berry development: i) maximum fresh seed weight and seed size coincided with the ending of the lag phase and the beginning of berry colouring ii) maximum dry seed weight and complete formation of seed features coincided with maximum berry weight.

- The number of seeds per berry, fresh and dry seed weight and berry weight were more affected by seasons than by different light conditions.
Chapter 4 - Developmental changes in phenolic composition of seeds and skins

4.1. Introduction
This study was aimed at investigating developmental changes in phenolic compounds in berry skin (total anthocyanins and total skin phenolics) and phenolic compounds in seeds (flavan-3-ol monomers and procyanidins (tannins)) during seed and berry development. The flavan-3-ol monomers studied were catechin (C), epicatechin (EC) and epicatechin gallate (ECG). The term seed tannin includes procyanidins which are polymers comprised of flavan-3-ol terminal and extension subunits. Seed monomers and tannins are localised in the seed coat (Pratt 1971, Thorngate and Singleton 1994) and it has been suggested that in order to increase impermeability of the seed coat, they act as a biochemical barrier to the permeability to oxygen (Werker 1980/81, 1997). This is based on an assumption that oxidation of the phenolic compounds reduce the oxygen available to the embryo. It is also widely accepted that seed tannins are responsible for the colour of the seed coat.

The major objective in this study was to examine developmental changes in phenolic composition of seeds and skins and to investigate possible relationships between them in relation to time and in the way these changes occurred.

4.2. Materials and methods

4.2.1. Seed sample preparation
Survey of solvents and methods for the extraction of seed phenolics
In various studies of seed tannins a range of solvents have been used for their extraction: ethyl acetate (Czochanska et al. 1979, Salagoity-Auguste and Bertrand 1984, Yokotsuka and Singleton 1987), ethyl acetate and then chloroform (Oszmianski and Sapis 1989), acetone and ethyl acetate (Boukharta et al. 1994), as well as the mixtures of various solvents with water in different proportions such as: methanol/water (75:25), acetone/water (70:30) (Kallithraka et al. 1995), acetonitrile/water (75:25) (Oszmianski and Sapis 1989, Ricardo da Silva et al. 1990), or ethyl acetate/water (90:10) (Pekic et
at. 1998). The preparation of the seeds also varied and the extractions selected for specific purposes, were performed on whole seeds, ground or homogenised seeds. In order to determine the most suitable solvent, a study was conducted to investigate the extraction efficiencies of different solvents such as 70% v/v aqueous acetone, 70% v/v aqueous ethanol and 90% v/v aqueous ethyl acetate using whole seeds and homogenised seeds. The experiment was carried out on Shiraz seeds collected at Nuriootpa in 1998. Seeds from frozen berries were separated from flesh under liquid nitrogen, weighed, counted and used immediately for the extraction. Nine replicate samples were analysed.

**Extraction of whole grape seeds**

Whole grape seeds (1 g) were extracted with 20 mL of either 70% v/v aqueous ethanol, 70% v/v aqueous acetone or 90% v/v aqueous ethyl acetate in sealed tubes, at room temperature, during the period of 24 hours. The organic solvents were removed by evaporation under reduced pressure and also under nitrogen gas cover at 30°C and the residue was dissolved in 5 mL 100% methanol and filtered through a 0.45 μm membrane filter. The content of monomers and seed tannins was determined by the reverse phase HPLC method described below (Peng et al. 2001).

**Extraction of homogenised grape seeds**

Grape seeds (1 g) were homogenised with 20 mL of one of the following solvents 70% v/v aqueous ethanol, 70% v/v aqueous acetone and 90% v/v aqueous ethyl acetate using a high speed homogeniser. The extraction was carried out by placing samples on a rotary wheel during 1 hour. The samples were centrifuged and the supernatant and the precipitate separated. The solvents were evaporated to dryness under reduced pressure and nitrogen gas cover, at 30 °C. The residue was dissolved in 5 mL of 100% methanol and filtered through a 0.45 μm membrane filter. The content of monomers and tannins was determined by HPLC (Peng et al. 2001).

**Results of the seed extraction experiment**

The experiment showed that the yield of the extracted phenolic compounds was dependent on solvent type and the method of extraction. Extraction of whole seeds with acetone gave 50% and 97% higher yield of total tannins and 9% and 7% higher yield of total monomers compared to the extractions with ethanol and ethyl acetate respectively. Although the method of homogenising seeds enhanced the extraction of monomers and
seed tannins using both acetone and ethanol, homogenising or grinding of seeds may cause a significant increase in the extraction of additional compounds from grape seeds (Pekic et al. 1998). The problem of “carry over” peaks in the chromatograms between samples may be explained by these undesired compounds. Ethyl acetate was recommended as the best solvent for the extraction of phenolic compounds of lower molecular weight (Pekic et al. 1998) but in this current investigation, it showed very poor extraction abilities accompanied by a chromatogram with broad and overlapped peaks. Based on the above results, it was decided to use 70% v/v aqueous acetone for the extraction of whole seeds in order to extract as high yield of seed tannins as possible and to avoid interference from additional undesirable compounds which usually occurred when using homogenised seeds.

**Analysis of flavan-3-ol monomers and tannins**

Seeds from one sub-sample were used for the determination of monomer flavan-3-ols and seed tannins. Seeds were counted, weighed and kept at -80°C until analysed. Frozen seeds were placed in 50 mL 70% v/v aqueous acetone and extracted over 24 hours with mixing at room temperature. Acetone was removed under reduced pressure at 35°C and the residual aqueous solution diluted to 25 mL with distilled water. Ten mL of this solution was transferred to a sample tube, frozen and freeze-dried. The solid residue was dissolved in 5 or 10 mL methanol.

Analysis of the phenolic composition of seeds was by HPLC (Peng et al. 2001) and the phloroglucinol method (Kennedy and Jones 2001). Flavan-3-ol monomers were analysed according to the method of Peng et al. (2001). Prior to HPLC analysis one part of the methanol extract was diluted with nine parts of distilled water and filtered. The HPLC apparatus used was a standard Hewlett-Packard HP 1100 instrument equipped with a diode array detector. The reverse-phase column was an Alltima C18 analytical column, protected by a guard column containing the same material. The method utilises a binary gradient solvent system consisting of 0.2 % v/v aqueous phosphoric acid (mobile phase A) and 1:4 w/w mobile phase A/acetonitrile (mobile phase B). The elution conditions were as follows: 1.0 mL/min; linear gradient from 0 to 15% B in 15 min, 15 to 16% B in 25 min, 16 to 25 % B in 1 min, 25% B for 5 min, 25 to 60% B in 5 min, 60 to 100% B in 5 min, 100% B for 3 min and a linear gradient from 100 to 0% B in 1 min. The column was then conditioned for 5 min before the next injection. Full
spectral scans with retention times and absorbance at 280 nm were taken. Eluting peaks were identified by retention time and spectral comparison to known standards of catechin, epicatechin and epicatechin gallate. Four different concentrations of each standard were used to construct the calibration curve. One sample of a standard of catechin was injected after each group of eight analysed samples. Flavan-3-ol monomers peaks were quantified using their eluting areas relative to a catechin standard. All chromatographic solvents were HPLC grade. Catechin, epicatechin, epicatechin-gallate and ascorbic acid were purchased from Sigma (Castle Hill, NSW, Australia).

Seed tannin subunits were analysed according to the method by Kennedy and Jones (2001). Acid catalysed cleavage of the extract of seed tannins in the presence of excess phloroglucinol released the pool of terminal and monomer subunits and the pool of extension subunits. The concentration of terminal subunits was determined by subtracting the concentration of free monomers in the uncleaved solution from that of the cleaved solution. The concentration and structure of extension subunits was determined from the pool where the interflavan bond was replaced with a phloroglucinol moiety at the 4-position of the C-ring (Kennedy and Jones 2001). The mean degree of polymerisation was determined by dividing the amount of total subunits (terminal and extension subunits) by the terminal subunit amount. Prior to HPLC analysis the methanol extract was diluted (1:1) with a solution containing 0.2 M HCl, 100 g/L phloroglucinol and 20 g/L ascorbic acid in methanol. The mixture was reacted at 50°C for 20 min, and then combined with 5 volumes of 40 mM aqueous sodium acetate. The samples were filtered and analysed on a standard Hewlett-Packard HP 1100 instrument (HPLC) equipped with a diode array detector. The column was a Wakosil II 5 C 18 analytical column (250 x 4.6mm, particle size 5 mm) purchased from SGE, protected by a guard column containing the same material. The method utilised a binary gradient with mobile phase containing 1% v/v aqueous acetic acid (mobile phase A) and methanol (mobile phase B). Elution conditions were as follows: 1.0 mL/min; 5% B for 10 min, linear gradients from 5 to 20% B in 20 min, and 20 to 40% B in 25 min. The column was washed with 90% B for 10 min and re-equilibrated with 5% B for 5 min before the next injection. Eluting peaks were monitored at 280 nm, identified by their relative retention time and quantified using their absorbance spectra relative to a catechin standard. Four different concentrations of a catechin standard were used to
construct the calibration curve. One sample of the catechin standard was injected after each group of eight analysed samples. The concentration of extension subunit-phloroglucinol adducts was calculated from published molar extinction coefficients (Kennedy and Jones 2001). Phloroglucinol and sodium acetate were purchased from Aldrich (Castle Hill, NSW, Australia).

4.2.2. Berry sample preparation
Juice total soluble solids and pH were measured on the juice, obtained by crushing a sub-sample of 50 fresh berries, using a refractometer and a pH meter respectively. Five mL of centrifuged juice was diluted in 20 mL of distilled water, frozen and later, after thawing, titratable acidity (TA) was determined by an automatic compact titrator (Crison, Version III, Spain). TA was expressed as g/L of tartaric acid.

The concentration and level of total anthocyanins and total skin phenolics were determined according to the method of Iland et al. (2000). Weight of whole berries was taken and then seeds removed, counted and weighted. The berry skin and flesh were homogenised using a “Janke and Kunkel” Ultra-turrax T25 homogeniser at 24000 rpm for approximately 1 minute each. One gram of a homogenate was extracted with acidified 50% ethanol (pH 2) over one hour with mixing. After centrifugation, 1 mL of extract was mixed with 10 mL 1M HCl and left for 3 hours in a dark place. Absorbance of the extract was read at 520 nm and 280 nm using a “Carry-100” spectrophotometer in a 10 mm cuvette.

4.3. Results
4.3.1. Developmental changes in phenolic composition of seeds
4.3.1.1. Developmental changes in the concentration and level of total tannins
The pattern of developmental changes in the concentration (mg/g berry weight) and level (mg/berry) of seed total tannins showed that the majority of tannins was synthesised at the beginning of berry development (Figure 4.1). The highest level of seed tannins, observed when berries entered veraison, was 4.3-4.7 and 3.3-4.0 mg cat eq/berry (at approximately 64 and 56 DAF in the first and second season respectively). Thereafter the level of seed tannins sharply declined until 91-98 DAF (40% of
maximum), and then slowly declined, falling to 2.0-2.3 or 1.8-1.9 mg cat eq/berry (around 120 and 110 DAF in the first and second season respectively). The concentration of seed tannins followed a similar pattern and at approximately 91 DAF, it was 60% of that at the beginning of veraison and thereafter slowly declined to 1.5-1.6 and 1.3-1.5 mg cat eq/g berry weight (120 and 110 DAF in the first and second season respectively) (Figure 4.1).

The maximal concentration of seed tannins, expressed on a per gram seed weight basis, of 62-65 and 62-67 mg/g seed weight was observed at approximately 64 and 56 DAF in the first and second season respectively, and then sharply declined by 43-48% to 36-37 and 32-33 mg/g seed weight (120 and 110 DAF in the first and second season respectively) (data not shown).

![Figure 4.1](image_url)

Figure 4.1. Developmental changes in the concentration and level of seed tannins during berry development and ripening under different light conditions. The berries of the following treatments: ♦ ST (1-10% sunlight at the bunch zone), ■ MET (10-50% sunlight at the bunch zone) and △ HET (40-80% sunlight at the bunch zone) were sampled during (a) 1999/2000 and (b) 2000/2001 season. Each point represents the mean of eight replicates and error bars represent ± standard error of the mean.
Differences between treatments and seasons
The pattern of developmental changes in the concentration and level of seed tannins was similar for all the treatments with some variations during veraison which maybe due to the difference in berry size between treatments as well as different proportions of green and red coloured berries in the sample (Figure 4.1). After approximately 91 DAF there were no significant differences (P<0.001) between treatments in the concentration and level of seed tannins. The level of seed tannins (per berry or per seed) was 20% higher in the first than second season due to significant differences (P<0.001) in the number of seeds per berry (1.75 and 2.50 in the first and second season respectively), seed weight and berry weight (30 and 20% greater in the first season than in the second season), but the concentration of seed tannins, expressed on a per gram berry weight or per gram seed weight basis, was similar in both seasons.

4.3.1.2. Developmental changes in the concentration and level of extension and terminal subunits
The extension subunits represented more than 90% of seed total tannins and terminal subunits less than 10%. The patterns of developmental changes in the concentration and level of extension subunits were the same as those for total seed tannins (Figure 4.2). During the period between the beginning of veraison and approximately 92 DAF the concentration of extension subunits declined from 4.2 and 4.0 to 1.5 and 1.4 mg cat eq/g berry weight (in the first and second season respectively), which represented a decline of 60-65 %, but in next 2-3 weeks the decline was only 10%. In the same period the level of extension units declined from 3.2 and 2.7 to 2.3 and 1.7 mg cat eq/berry (91-92 DAF) and then to 1.9 and 1.5 mg cat eq/berry (approximately 110 DAF) (in the first and second season respectively).
Figure 4.2. Developmental changes in the concentration and level of extension subunits in seeds during berry development and ripening under different light conditions. The berries of the following treatments: ✓ ST (1-10% sunlight at the bunch zone), □ MET (10-50% sunlight at the bunch zone) and ▲ HET (40-80% sunlight at the bunch zone) were sampled during (a) 1999/2000 and (b) 2000/2001 season. Each point represents the mean of eight replicates and error bars represent ± standard error of the mean.

Figure 4.3. Developmental changes in the composition of extension subunits in seeds during berry development and ripening under different light conditions. The berries were sampled during (a) 1999/2000 and (b) 2000/2001 season. Each point represents the mean of 24 replicates and error bars represent ± standard error of the mean. C is catechin, EC is epicatechin and ECG is epicatechin gallate.
The composition of extension units was relatively constant through the development period with the ratio of catechin (C): epicatechin (EC): epicatechin gallate (ECG) averaging 5:56:39 and 5:54:41 in the first and second season respectively (Figure 4.3).

The concentration and level of terminal subunits in seeds increased from approximately 40 DAF and reached the maximal values of 1.7 mg cat eq/g berry weight or 1.3-1.4 mg cat eq/berry and 1.1-1.6 mg cat eq/g berry weight or 0.8-1.0 mg cat eq/berry at approximately 64 and 56 DAF in the first and second season respectively (Figure 4.4). After veraison the concentration of terminal units sharply declined to 0.5 and 0.3 mg cat eq/g berry weight at approximately 91-92 DAF which represented a decrease of 70%. Further decline of 10-15% was observed in the next 2-3 weeks (until the end of the sampling period). The level of terminal units had a similar pattern with a decrease of 60% until 91-92 DAF and further 10-20% until 110-120 DAF.

![Figure 4.4. Developmental changes in the concentration and level of terminal subunits in seeds during berry development and ripening under different light conditions. The berries of the following treatments: ❧ ST (1-10% sunlight at the bunch zone), ■ MET (10-50% sunlight at the bunch zone) and △ HET (40-80% sunlight at the bunch zone) were sampled during (a) 1999/2000 and (b) 2000/2001 season. Each point represents the mean of eight replicates and error bars represent ± standard error of the mean.](image-url)
The major constituent of terminal subunits was epicatechin gallate while the proportion of catechin and epicatechin changed during the period of berry development and ripening. Prior to veraison as the proportion of catechin decreased, the proportion of epicatechin increased (Figure 4.5). After veraison the proportion of both catechin and epicatechin increased until they both represented around 20-25% of total terminal subunits.

![Figure 4.5](image.png)

**Figure 4.5.** Developmental changes in the composition of terminal subunits in seeds during berry development and ripening under different light conditions. The berries were sampled during (a) 1999/2000 and (b) 2000/2001 season. Each point represents the mean of 24 replicates and error bars represent ± standard error of the mean. C is catechin, EC is epicatechin and ECG is epicatechin gallate.

**Differences between treatments and seasons**

In the later stages of berry development and ripening there was no significant difference ($P<0.001$) in the concentration and level of extension and terminal subunits in seeds of berries developed and ripened under different sunlight intensity at the bunch zone. Different light conditions had no effect on the composition of the subunits and all the treatments had similar proportions of catechin, epicatechin and epicatechin gallate in the extension and terminal subunits. When compared between seasons, the level of extension (20%) and terminal subunits (50%) was greater in the first than second season, but expressed on a per gram berry weight or per gram seed weight, the concentration of extension subunits was similar in both seasons, while the concentration of terminal subunits was greater by 60% in the first than in the second season.
4.3.1.3. Developmental changes in the concentration of total flavan-3-ol monomers

The flavan-3-ol monomers in seeds included catechin (C), epicatechin (EC) and epicatechin-gallate (ECG). The observed pattern of developmental changes in the concentration of total free flavan-3-ol monomers increased until the maximum of 1.4-1.5 and 1.3-1.4 mg/g berry weight was reached at veraison (64 DAF in the first and second season respectively) and then sharply declined to 0.5 and 0.15 mg/g berry weight at approximately 91 DAF (in the first and second season respectively) (Figure 4.6). Thereafter the concentration of monomers declined further to 0.3 and 0.1 mg/g berry weight in the first and second season respectively. The level of monomers followed the same pattern, with a maximum of 1.2-1.4 and 1.0 at 64 DAF after which declined to 0.4-0.5 and 0.2 mg/berry approximately 110 DAF in the first and second season respectively) (Figure 4.6).

Figure 4.6. Developmental changes in the concentration and level of flavan-3-ol monomers in seeds during berry development and ripening under different light conditions. The berries of the following treatments: ♦ ST (1-10% sunlight at the bunch zone), ♦ MET (10-50% sunlight at the bunch zone) and △ HET (40-80% sunlight at the bunch zone) were sampled during (a) 1999/2000 and (b) 2000/2001 season. Each point represents the mean of eight replicates and error bars represent ± standard error of the mean.
The maximal concentration of flavan-3-ol monomers, expressed on a per gram seed weight basis, observed at approximately 73 DAF, was 19-20 and 13-14 (in the first and second season respectively. Thereafter the concentration of monomers declined by approximately 70% and was 6.4-6.9 and 2.5-2.8 mg/g berry weight (110-120 DAF in the first and second season respectively).

The proportional composition of monomers changed during the period of berry development and ripening and while the proportion of epicatechin steadily increased to 70% of total monomers, the proportion of catechin decreased to 25% (Figure 4.7). The proportion of epicatechin gallate decreased from 7% (around veraison) to 2-4% at the end of the sampling period (Figure 4.7).

![Figure 4.7. Developmental changes in the composition of flavan-3-ol monomers in seeds during berry development and ripening under different light conditions. The berries were sampled during (a) 1999/2000 and (b) 2000/2001 season. Each point represents the mean of 24 replicates and error bars represent ± standard error of the mean. C is catechin, EC is epicatechin and ECG is epicatechin gallate.](image)

**Differences between treatments and seasons**

The patterns of developmental changes in the concentration and level of free monomers was similar for all treatments except the maximal level of monomers for ST was reached at 73 DAF in the first season (7 days later than MET and HET). In both seasons, after veraison, at each sampling date there was no significant difference ($P<0.05$) between treatments in the concentration and level of free monomers except in the first season, at 113 DAF, ST was significantly higher ($P<0.001$) when compared to MET and HET. Sunlight intensity at the bunch zone had no effect on the pattern of
changes of the proportional composition of free monomers. Both the concentration and level of flavan-3-ol monomers was significantly higher in the first than second season.

4.3.1.4. Developmental changes in polymer size (mean degree of polymerisation)

At the beginning of berry development, due to a high level of extension subunits, the polymer size (mDP) was around 8 subunits. As the level of terminal subunits increased towards veraison, polymer size decreased to around 5 subunits and thereafter with the level of both extension and terminal units declining, polymer size remained at the level of around 4-5 and 5-6 subunits (in the first and second season respectively) (Figure 4.8).

![Figure 4.8](image)

Figure 4.8. Developmental changes in polymer size of seed tannins during berry development and ripening under different light conditions. The berries of the following treatments: • ST (1-10% sunlight at the bunch zone), ■ MET (10-50% sunlight at the bunch zone) and △ HET (40-80% sunlight at the bunch zone) were sampled during (a) 1999/2000 and (b) 2000/2001 season. Each point represents the mean of eight replicates and error bars represent ± standard error of the mean.

Differences between treatments and seasons

Through the developmental period, until approximately 91 DAF, higher variation in the difference in polymer size (mDP) between treatments was observed, but the variation declined towards the end of the sampling period (Figure 4.8).
4.3.2. Developmental changes in the chemical composition of berries

4.3.2.1. Developmental changes in juice total soluble solids (°Brix)

In both seasons through the period of berry development and ripening, at each sampling date, the sugar concentration expressed as juice total soluble solids (°Brix), was lower in the juice of ST berries compared to MET and HET treatments. In the first season the difference was significant (P<0.001) in the period between post veraison and approximately 106 DAF, while in the second season the difference was consistent through the ripening period (Figure 4.9). The trend of changes for MET and HET indicated similarity of the two treatments.

![Figure 4.9](image)

Figure 4.9. Developmental changes in juice total soluble solids (°Brix) during berry development and ripening under different light conditions. The berries of the following treatments: ♦ ST (1-10% sunlight at the bunch zone), ■ MET (10-50% sunlight at the bunch zone) and ▲ HET (40-80% sunlight at the bunch zone) were sampled during (a) 1999/2000 and (b) 2000/2001 season. Each point represents the mean of eight replicates and error bars represent ± standard error of the mean.

In both seasons, when the TSS concentration of 24-25 °Brix (the predetermined harvest level for winemaking) was reached in the berries of MET and HET, the berries of ST had a lower °Brix and the fruit was left to ripen for an additional 7 days and then harvested for winemaking, so that the °Brix levels of samples from all treatments were similar for the winemaking (see Chapter 6).
4.3.2.2. Developmental changes in the concentration and level of total anthocyanins

During the period of berry ripening, at the same sampling date, the concentration and level of total anthocyanins was significantly lower in skins of the ST treatment, while berries of MET and HET treatments were similar (Figure 4.10.a). The maximal concentration and level of total anthocyanins in berries of all treatments was reached at approximately 113 DAF in the first season. The maximal concentration of total anthocyanins was 1.31, 1.46 and 1.47 mg/g berry weight while the maximal level of anthocyanins was 1.71, 1.88 and 2.00 mg/berry for ST (24.3 °Brix), MET (24.9 °Brix) and HET (24.9 °Brix) respectively, indicating that ST was significantly (P<0.001) lower than MET and HET, while there were no significant difference between MET and HET (Figure 4.10.a).

In the second season the maximal concentration and level of total anthocyanins was reached at approximately 110 DAF for MET (24.1 °Brix) and HET (24.2 °Brix) and at 117 DAF for ST (25.0 °Brix). Although the ST treatment reached higher TSS, the concentration of total anthocyanins for ST (1.23) was significantly lower than MET (1.58) and HET (1.51 mg/g berry weight) as well as the level of total anthocyanins (1.29, 1.59 and 1.56 mg/berry for ST, MET and HET respectively). There was no significant difference in the concentration and level of total anthocyanins between MET and HET.
The concentration (mg/g berry weight) of total anthocyanins was plotted against TSS to enable the comparison between treatments at similar maturity stage (°Brix) (Figure 4.10.b). In general, developmental changes in the concentration of total anthocyanins in relation to TSS, showed an increasing trend with the increase of TSS (°Brix) until the maximum concentration was reached, and thereafter declined (Figure 4.10.b). Their relationship was defined as a highly significant ($P<0.001$) positive polynomial relationship with the coefficient of determination ($r^2$) of 0.96 and 0.98 for all treatments in the first and second season respectively. A comparison of fitted curves showed that within the same season the pattern of changes was similar for each treatment but the slopes of curves differed in relation to the effect of different light conditions on the concentration of total anthocyanins.
Figure 4.10.b. Developmental changes in concentration and level of total anthocyanins of berries ripened under different light conditions in relation to changes in total soluble solids (°Brix). The berries of the following treatments: ♦ ST (1-10% sunlight at the bunch zone), ♣ MET (10-50% sunlight at the bunch zone) and Δ HET (40-80% sunlight at the bunch zone) were sampled during (a) 1999/2000 and (b) 2000/2001 season. Each point represents the mean of eight replicates and error bars represent ± standard error of the mean. Polynomial curves are fitted for each treatment.

The highly significant (P<0.001) positive polynomial relationship between the changes in the level (mg/berry) of total anthocyanins and TSS is shown in Figure 4.10.b. The coefficient of determination ($r^2$) was 0.92-0.94 and 0.96-0.96 for all treatments in the first and second season respectively. Similar to the concentration of total anthocyanins, the slopes of fitted curves for the level of total anthocyanins differed between treatments, but the curve shapes were similar (Figure 4.10.b).
Differences between treatments and seasons

During the whole ripening period, at the same stage of maturity (°Brix), the concentration and level of total anthocyanins in berries that were grown under reduced sunlight intensity (ST) was lower compared to berries grown under higher sunlight intensity (MET and HET). In the first season the differences were significant (*P*<0.001) only during the period of 20-26 °Brix, while in the second season the difference was consistent throughout the ripening period (Figure 4.10.b). When comparing MET and HET the concentration and level of total anthocyanins was similar in the first season, while in the second the level was slightly higher for MET due to lower berry size of MET (Figure 4.10.a and Figure 4.10.b).

4.3.2.3. Developmental changes in the concentration and level of skin total phenolics

During the period of berry ripening the concentration and level of total skin phenolics was significantly lower in skins of the ST treatment when compared to MET and HET treatments (Figure 4.11.a). The minimal concentration of skin total phenolics was observed at veraison (64-73 DAF) and it was 0.21, 0.32 and 0.33 au/g berry weight and 0.13, 0.18 and 0.24 au/g berry weight for ST, MET and HET in the first and second season respectively. At the same time, the minimal level of skin phenolics was 0.16, 0.23 and 0.24 au/berry and 0.09, 0.12 and 0.16 au/berry for ST, MET and HET in the first and second season respectively (Figure 4.11.a).

In the first season the maximum concentration of skin total phenolics of 0.74 (ST), 0.83 (MET) and 0.87 au/g berry weight (HET) was reached at 120 DAF (at TSS value of 25.6, 25.3 and 25.4 °Brix for ST, MET and HET respectively). At the same time the maximum level of skin total phenolics was 0.98, 1.07 and 1.22 au/berrie for ST, MET and HET respectively (Figure 4.11 a). In the second season the maximal concentration of skin phenolics was 0.73, 0.90 and 0.91 au/g berry weight, while the maximal level of skin phenolics was 0.77, 0.90 and 0.94 au/berry for ST, MET and HET respectively. These values were observed when berries reached TSS of 25.0 °Brix for ST (117 DAF) and 24.2 °Brix (MET) and 24.1 °Brix (HET) (110 DAF).
Figure 4.11.a. Developmental changes in concentration and level of skin total phenolics during berry ripening under different light conditions. The berries of the following treatments: • ST (1-10% sunlight at the bunch zone), ■ MET (10-50% sunlight at the bunch zone) and Δ HET (40-80% sunlight at the bunch zone) were sampled during (a) 1999/2000 and (b) 2000/2001 season. Each point represents the mean of eight replicates and error bars represent ± standard error of the mean.

The concentration and level of total skin phenolics were plotted against total soluble solids for each treatment sampled during the ripening period (Figure 4.11.b). The relationship between the concentration of total skin phenolics and TSS was defined as a positive polynomial relation with a coefficient of determination ($r^2$) for each treatment being in the range of 0.85-0.88 and 0.69-0.90 for all treatments (in the first and second season respectively). Similarly the relationship between the level of total skin phenolics and TSS was a highly significant ($P<0.001$) polynomial relation with a coefficient of determination ($r^2$) for each treatment of 0.86 (for the first season) or in the range of 0.88-0.98 (for the second season).
The general trends of developmental changes in the concentration and level of total skin phenolics decreased towards veraison and then increased, following the increase in TSS (Figure 4.11.b). A comparison of fitted curves within the same season showed similar shapes, but different slopes for different treatments (Figure 4.11.b).

**Differences between treatments and seasons**

In both seasons, at the same stage of ripeness (°Brix) the level and concentration of skin total phenolics was significantly lower \( (P<0.001) \) in skins of the ST treatment than MET and HET, while MET and HET had similar values (Figure 4.11.b).
4.3.3. Relationships between developmental changes in seeds and berries

The objective of this study was to investigate relationships between developmental changes in seeds and in skin components. The relationships between the seed coat colour value and i) the changes in the phenolic composition of berries (the level of total anthocyanins and the concentration and level of skin phenolics) and ii) the changes in phenolic composition of seeds (the level of seed tannins) were defined as polynomial relationships (Figure 12). During the period of seed growth and development the colour of the seed coat underwent changes from green, through yellow to brown (as described in Chapter 3) which may be a reflection of the changes in the phenolic composition of seeds. In general, as the concentration of extracted seed tannins decreased, seeds were a darker brown. At the same time berries accumulate higher concentration of anthocyanins and skin total phenolics. The established relationships between changes in the phenolic composition of seeds and changes in the phenolic composition of skins indicated that these changes occurred simultaneously and that they were related to berry maturity.
Figure 4.12. The relationships between the seed coat colour value and the level of seed tannins, the level of total anthocyanins, the level of skin total phenolics during the period from the beginning of veraison until harvest using data from all treatments. Significance of the coefficient of determination indicated by *** is significant at $P<0.001$. Note the different y-axis scales.
4.4. Discussion

4.4.1. Developmental changes in the phenolic composition of seeds and their relationships with seed and berry development

In small green seeds (28 DAF) the concentration of seed tannins was at its maximum indicating that the accumulation of seed tannins commenced with the early stage of seed development. When expressed on a per seed or per berry basis, the level of seed tannins showed a peak at the beginning of veraison (64 and 56 DAF in the first and second season). This is in agreement with other studies that reported that the bulk of tannin synthesis occurred prior to veraison (Romeyer et al. 1986, Katalinic and Males 1997, Kennedy et al. 2000a, Kennedy et al. 2000b, Jordao et al. 2001, Downey et al. 2003a). After veraison, until approximately 91 DAF, the concentration and level of seed tannins sharply decreased by 60% and thereafter the decrease was at a slower rate. The trend of changes in the extension and terminal subunits followed the same pattern. The concentration and level of monomer flavan-3-ols increased towards veraison, with a peak at around 64 DAF in both seasons, and then decreased (Figure 4.2, 4.3, 4.4). The decline of the seed tannins was attributed to their reduced extractability (Saint-Cricq de Gaulejac et al. 1997, Kennedy et al. 2000b). Downey et al. (2003a) reported that the yield of tannins in the remaining residue after extraction increased post veraison. The sum of extractable and residue derived tannins represented 80% of the maximal value observed around veraison, supporting the hypothesis that the decrease of extractable tannins may be attributed to the formation of branched or oxidatively linked polymers (Ricardo da Silva et al. 1991a, Saint-Cricq de Gaulejac et al. 1999a, Saint-Cricq de Gaulejac et al. 1999b).

The major constituent of extension units was epicatechin (Figure 4.3), while epicatechin gallate was the most abundant in terminal units (Figure 4.5) and epicatechin in the pool of free monomers (Figure 4.7). The patterns of changes in the composition and in the concentration and level of seed total tannins and free monomer flavan-3-ols were similar to these previously reported (Romeyer et al. 1986, Saint-Cricq de Gaulejac et al. 1997, de Freitas et al. 1998, Kennedy et al. 2000a, Kennedy et al. 2000b, Jordao et al. 2001, Downey et al. 2003a).
Relationship between developmental changes in the phenolic composition of seeds and seed development
The results from this study indicated a relationship between the changes in the phenolic composition of seeds to the particular sequences of seed development (Figure 14). As described in Chapter 3 seed growth and development was divided into the following phases: a phase of seed growth, an intermediate phase and a phase of seed drying and maturation. The biosynthesis of seed tannins commenced at the beginning of the first phase. The maximum level of seed tannins was reached at the beginning of the second phase while the maximal level of monomers was reached at the end of the second phase of seed development. During the third phase of seed development a sharp decrease in the level of seed tannins followed the same trend as a decline in water content and was completed at the point of maximum dry seed weight, the plateau of the difference between fresh and dry seed weight and completion of development of all the seed features.

Relationship between developmental changes in the phenolic composition of seeds and berry development and ripening
The results indicated that the sequence of changes in the level of seed tannins and monomers coincided to the sequences of berry development and ripening. The maximum level of seed tannins was observed at the beginning of berry colouring (veraison) while the maximum level of flavan-3-ol monomers coincided with the end of veraison. Furthermore, a sharp decline in the level of extractable seed tannins ended at the time berries reached their maximum weight (Figure 4.13).

Developmental changes in phenolic composition of seeds and skins occurred simultaneously and highly significant ($P<0.001$) relationships established between the seed coat colour value and the level of total tannins in seeds indicated that i) the seed coat colour may be an indicator of seed maturity and ii) a lower amount of seed tannins would be extracted from seeds that were darker in colour and harder in texture (Figure 13). The strong relationships between i) the seed coat colour value and the level of seed tannins and ii) and the phenolic composition of berries (the level of total anthocyanins and skin total phenolics indicated that developmental changes in seeds occurred simultaneously with developmental changes in berries (Figure 4.13).
Figure 4.13. Notional diagram of seed and berry development showing how the sequences of seed development coincide with the sequences of berry development: i) seed formation and berry formation were completed by the beginning of veraison when the maximum level of seed tannins and maximal fresh seed weight were observed (60 DAF) ii) the level of monomers was at a maximum and the rapid oxidation of seed tannins commenced at veraison (70 DAF) iii) formation of all seed features (the beak, chalaza and raphe) was completed, dry seed weight was at a maximum, rapid oxidation of seed tannins finished and berries reached their maximum weight (92 DAF).
A practical implication of this study is that the value of the seed coat colour can be easily assessed visually at the later stages of berry development, which is not possible for the dark colour of berry skin. This suggests that the seed coat colour can be used as an indicator of overall seed maturation and berry ripeness. However it is important to note that berries of different treatments can reach the same maturity, in terms of TSS (°Brix) and have a similar value for the seed coat colour, but the concentration of anthocyanins in berry skin and/or the concentration of seed tannins can be significantly different between treatments and these concentrations need to be assessed using the analytical methods.

4.4.2. Hypothesis about oxidation of seed tannins
The objective of this study was to examine the changes in phenolic composition of seeds and also to investigate relationships with seed development over time. It was also important to consider possible mechanisms for these changes and relationships. The following section i) discusses how the findings of this study contribute and relate to knowledge of mechanisms of seed development reported in the literature and ii) develops an hypothesis which connects the data of this study and the mechanisms of seed development.

The bulk of procyanidins are synthesised at the early stages of seed development in vesicles budded off from the endoplasmatic reticulum and later the vesicles coalesce and incorporate into the large central vacuole (Stafford 1988). During the disintegration of the cytoplasm and ruptures of vacuoles, procyanidins may easily come in contact with reactive oxidative species (ROS), enzymes (polyphenoloxidases and peroxidases) or metal ions and form some oxidation complexes among themselves, but very often those complexes involve the co-substrate, such as proteins (Haslam 1998) or polysaccharides (Fry and Miller 1989). ROS are by-products formed as a result of successive on-electron reduction of molecular oxygen (O₂) and include: the superoxide radical (O₂)⁻⁺, hydrogen peroxide (H₂O₂) and the hydroxy radical (OH). The level of ROS is maintained at a particular level by enzymes, but its production is regulated by light, hormones, dehydration or wounding (Bolwell and Wojtaszek 1997). Under stress conditions cells are able to produce an excessive amount of ROS that becomes a key player in programmed cell death (PCD) (Jabs 1999). Many studies on cereal aleurone
showed that PCD of aleurone cells was mediated by ROS and regulated by hormones (Bethke et al. 2001, Bethke and Jones 2001, Fath et al. 2001a). Gibberelic acid (GA) and abscisic acid (ABA) are involved in PCD and while GA may induce PCD, ABA may prevent PCD by producing the high level of enzymes that would scavenge ROS (Bewley 1997, Fath et al. 2001b). Studies by Ricardo da Silva et al. (1991a) and Saint-Cricq de Gaulejac et al. (1999b) have shown that phenolic compounds may scavenge active oxygen species giving extremely reactive o-quinones that could lead to oxidation and polymerisation reactions of phenolics. Another group of substances that may react with phenolics during raptures of vacuoles are enzymes polyphenoloxidases (PPO) and peroxidases (POD). It is commonly accepted that the enzymes PPO and POD are involved in oxidative degradation of phenolics compounds which lead to browning. PPO is the enzyme which catalyses two different reactions in the presence of molecular oxygen: the hydroxylation of monophenols to o-diphenols and the oxidation of o-diphenols to o-quinones. Peroxidase catalyses single-electron oxidation and it has been shown that this is correlated with biosynthesis of lignins (Goldberg et al. 1985, Stafford 1988). These enzymes in grapes have been described by many authors (Kidron et al. 1978, Wissemann and Lee 1980, Sapis et al. 1983), but neither provide definite evidence of enzyme involvement nor were any correlation between activity of enzymes and the concentration of procyanidins or total phenolic compounds found (Stafford 1989). This suggests that there may be additional ways in which phenolic compounds are oxidised. Based on the numerous studies of the effects of ROS on PCD in cereals aleurone it is hypothesised that upon desiccation, seed coat cells produced ROS which caused PCD. Phenolic compounds scavenged ROS, and activated by this process, reacted in self-polymerisation reactions or in cross-linking reactions with proteins. This would result in hardening of the seed coat, increasing impermeability of the seed coat, changes in the seed coat colour and lower extractability of phenolic compounds.

The first phase in seed development (0-60 DAF) was determined by rapid growth and development of seeds. During this time the flavan-3-ols and procyanidins accumulated. In the second phase (60-70 DAF) the fundamental physiological changes in seeds started. Rumination of fossettes and changes of cells around chalazal region were prominent, and difference between fresh and dry seed weight commenced. The accumulation of flavan-3-ols reached maximum. The peak activity of PPO at this stage, reported in studies of Kidron et al. (1978), Wissemann and Lee (1980), and Sapis et al.
(1983) is another interesting coincidence in seed development, but the involvement of PPO is still to be investigated. As flavan-3-ol monomers are more susceptible to oxidation to quinones compared to larger procyanidins chains, they may react as the initial substrate and subsequently oxidise more complex procyanidins (Stafford 1989). A possible role of PPO may be oxidation of the bulk of that initial material. Another role may be to maintain the level of ROS until all phenolic compounds are synthesised. The high concentration of ABA at veraison in berries (Coombe 1960, Coombe and Hale 1973, Scienza et al. 1978, Davies et al. 1997), and in seeds (Coombe and Hale 1973), indicates that its role may be correlated not only with the onset of berry ripening, but also with the onset of seed dormancy. It may be speculated that GA triggers PCD in the lag phase of a berry development, and then ABA produce high activity of PPO which would maintain the level of ROS until all phenolics material is synthesised. After veraison both the activity of PPO and the content of ABA decrease. After veraison (70-92 DAF) the seed coat hardened and progressively changed colour to dark brown. The formation of the seed features and rumination of fossettes, and difference between fresh and dry seed weight was accomplished by 92 DAF. Extractability of procyanidins declined rapidly indicating that the majority of PCD and oxidation of phenolic compounds were completed. This period was defined as programmed oxidation (Kennedy et al. 2000b). Thereafter, 92-120 DAF, seeds appeared to be dark brown. There was a small decline in the amount of extracted procyanidins. Radical signal measured by electron paramagnetic resonance (EPR) in the study of Kennedy et al. (2000b) showed a slow decrease, but still high value at the late stage of berry ripening. This indicated that PCD, release of ROS and oxidation of phenolics compounds were still occurring, but at a slower rate. Changes in the colour of chalaza, hilum and raphe indicated these cells as possible locations of slow oxidation of phenolic compounds. These cells were the last to die in the process which leads to a seed coat impermeability and seed dormancy.
4.4.3. Influence of sunlight intensity at the bunch zone on the phenolic composition of seeds and berries

Influence of sunlight intensity at the bunch zone on the phenolic composition of seeds

The concentration of seed tannins plotted against time (DAF) (Figure 4.1) enabled determination of changes in relation to sequences of seed and berry development, while plotting against TSS (Figure 4.14) enabled a comparison between treatments at similar stage of maturity (°Brix). The relationship between the changes in the concentration of seed tannins and berry ripening (the changes in TSS) was defined as a highly significant polynomial relationship with the coefficient of determination ($r^2$) being 0.98 and 0.96 (in the first and second season respectively) (Figure 4.14).

Figure 4.14. Developmental changes in the concentration and level of seed tannins in berries developed and ripened under different light conditions in relation to changes in total soluble solids (°Brix). The berries of the following treatments: · ST (1-10% sunlight at the bunch zone), = MET (10-50% sunlight at the bunch zone) and ∆ HET (40-80% sunlight at the bunch zone) were sampled during (a) 1999/2000 and (b) 2000/2001 season. Each point represents the mean of eight replicates and error bars represent ± standard error of the mean. Polynomial curves are fitted for each treatment.
The difference in the concentration and level of seed tannins between treatments at the same sampling date was prominent only around veraison but this may be due to the difference in the berry size or different proportions of green and red coloured berries in the sample which may result in various degrees of seed maturity and their respective phenolic composition. A comparison of fitted polynomial curves for each treatment showed no difference between treatments at later stages of berry maturity (Figure 4.14). Similarly, after approximately 91 DAF, there was no significant difference (P<0.05) between treatments in the concentration and level of extension and terminal subunits (data not shown). The concentration of seed monomers in berries grown under reduced light conditions showed some variation and was lower in the period of pre-veraison and higher post-veraison, than MET and HET at the same sampling date, which was a result of lower seed maturity and berry ripeness. When compared at the same stage of maturity there was no difference in the concentration of seed monomers between treatments (data not shown). The results clearly indicated that at later stages of berry maturity sunlight intensity at the bunch zone had no or little effect on the concentration, level and composition of flavan-3-ol monomers and seed tannins.

**Influence of sunlight intensity on total soluble solids (°Brix) and phenolic composition of berries**

At the same sampling date, total soluble solids in berries grown under reduced light conditions (ST) was significantly lower (P<0.001) than in berries of MET and HET. A fitted curve for ST had a more gradual slope indicating prolonged and delayed ripening (Figure 4.9). This is in agreement with various authors that reported that canopy and leaf shading compromised photosynthesis which resulted in lower total soluble solids (Kliwer 1970, Smart et al. 1985b, Reynolds et al. 1986, Rojas-Lara and Morrison 1989, Morrison and Noble 1990).

Different light conditions at the bunch zone significantly affected phenolic composition of berries including the concentration and level of total anthocyanins and skin total phenolics, supporting other studies that reported that biosynthesis and accumulations of anthocyanins and skin phenolics were dependent on light and temperature (Singleton et al. 1966, Buttrrose 1970, Kliwer 1977, Pirie and Mullins 1977, Crippen and Morrison 1986b, Rojas-Lara and Morrison 1989, Price 1994, Mabrouk and Sinoquet 1998, Spayd et al. 2002).
Reduced light conditions (<5% of ambient) significantly (P<0.001) decreased the concentration and level of total anthocyanins (Figure 4.10.a and Figure 4.10.b) and skin total phenolics in berries of ST (Figure 4.11.a and Figure 4.11.b). There was no significant difference in the concentration and level of anthocyanins in the berries grown under moderate sunlight intensity (10-40% of ambient) (MET) and berries grown under high light intensity (40-80% of ambient). A comparison of the fitted curves for the concentration of total anthocyanins at the same stage of ripeness (°Brix) (Figure 4.10.b) showed slightly higher slope for berries grown under moderate light intensity (MET) than for berries grown under high sunlight intensity (HET). A comparison of the fitted curves for the level of total anthocyanins at the same stage of ripeness (°Brix) (Figure 4.10.a) showed slightly higher slope for berries of HET than berries of MET which may be due to different berry size. Nevertheless, the results support the finding of Crippen and Morrison (1986b), Morrison and Noble (1990) and Mabrouk and Sinoquet (1998) that anthocyanin concentration was not linearly related to the bunch exposure, due to probably complex interactions between light and temperature effects on the bunch. The best fitted curves for the concentration and level of skin total phenolics indicated high dependency of the accumulation of skin total phenolics to intensity of sunlight (Figure 4.11.a and Figure 4.11.b) which is in agreement with other studies of Kliewer (1977) and Dokoozlian and Kliewer (1996). The results from this current study clearly indicated that the effects of sunlight intensity at the bunch zone are greater on skin than seed phenolic components. The influence of different light conditions on the phenolic composition of the berry skin will be discussed in detail in the next chapter.

The relationships between i) the concentration and level of total anthocyanins and TSS and ii) the concentration and level of skin total phenolics and TSS, were the best fitted by a polynomial equation (Figure 4.10.b and Figure 4.11.b) and the coefficient of determination indicated strong relationships between variables. Similar correlations were reported by various authors (Pirie and Mullins 1977, Wicks and Kliewer 1983), although Esteban et al. (2002) defined a linear correlation of the accumulation of total anthocyanins over the period from 12 °Brix to 23 °Brix. The results from this study would support the finding that the accumulation of phenolic compounds in the berry skin closely paralleled the accumulation of sugars. They also indicated that sunlight intensity affected the accumulation of phenolic compounds in the berry skin while the effect on the accumulation of sugars was reflected in delayed ripening.
4.5. CONCLUSION

- The maximum levels of tannins and flavan-3-ol monomers in seeds were observed at the beginning and the end of veraison respectively, after which both rapidly decreased. The major constituent of extension subunits was epicatechin (50-60%), while epicatechin gallate was the most abundant in terminal subunits (40-50%). The proportional composition of free monomers changes during development and as the proportion of catechin decreased to 25%, epicatechin increased to 70%.

- Developmental changes in the phenolic composition of seeds coincided with particular sequences in seed development. Biosynthesis of seed tannins commenced at the beginning of the first phase of seed growth and development. The beginning and the end of the second phase (intermediate phase) was marked by the maximal level of seed tannins and maximal level of seed monomers. During the third phase (a phase of seed drying and maturation), a sharp decrease in the level of extracted total seed tannins followed the same trend as a decline in water content and was completed at the point of maximum dry weight, the plateau of the difference between fresh and dry seed weight and completed development of all the seed features.

- Developmental changes in the phenolic composition of seeds coincided with particular sequences in berry development and ripening. The maximum level of seed tannins was observed at the beginning of veraison, the maximum level of flavan-3-ol monomers coincided with the end of veraison, while the end of the sharp decline in the level of seed tannins coincided with the point when berries reached their maximum weight.

- Changes of the seed coat colour were highly related to the changes in the concentration and level of seed tannins, total anthocyanins and skin total phenolics indicating that the external appearance and colour of the seed coat can be used as an additional indicator of seed maturity as well as overall berry ripeness.

- The relationships between changes in the phenolic composition of seeds and changes in the phenolic composition of skins indicated that these changes occurred simultaneously and that they were related to berry maturity.
- The strong inverse correlation between seed coat colour values and the concentrations of extracted seed monomers and tannins suggested that during development phenolics compounds underwent many chemical changes including oxidation. A hypothesis was developed about the possible mechanism of oxidation of phenolic compounds. Based on the evidence of this and other studies it is likely that oxidation of phenolic compounds is a part of programmed cell death, which is a natural sequence in seed maturation. Upon desiccation, seed coat cells produced ROS which caused PCD. Phenolic compounds scavenged ROS and during this process react in self-polymerisation reactions or in cross-linking reactions with proteins. This leads to increased impermeability of the seed coat and seed dormancy.

- Different light conditions affected accumulation of total soluble solids, the concentration and level of total anthocyanins and skin total phenolics, but had only a small effect on the concentration, level and composition of seed flavan-3-ol monomers and tannins.
Chapter 5 – The effects of sunlight intensity at the bunch zone on phenolic compounds in the skins

5.1. Introduction
Many studies have shown that the light environment of grape bunches is a critical factor in phenolic metabolism in the berry. Exposure to sunlight has been found to have both quantitative and qualitative effects on phenolic compounds in grape skins. Providing light energy (light effect) and heat (temperature effect) for bunches and leaves, sunlight influences both primary and secondary metabolism in grape berries. Many studies investigated the effects of sunlight intensity at the bunch zone on the concentration of phenolic compounds in berries (Kliewer 1970, Kliewer and Lider 1970, Roubelakis and Kliewer 1977, Bravdo et al. 1984, Smart et al. 1985b, Reynolds et al. 1986, Smart et al. 1988, Morrison and Noble 1990, Marais et al. 1992, Gao and Cahoon 1994, Price et al. 1995c, Dokoozlian and Kliewer 1996, Keller and Hrazdina 1998, Mabrouk and Sinoquet 1998, Haselgrove et al. 2000). In general, the authors reported that in cool climates the concentration of anthocyanins and skin phenolics increased with increasing sunlight intensity in the bunch zone, while in hot climates, the concentration of anthocyanins was not linearly related to the level of sunlight intensity and the maximum of anthocyanins was observed at a moderate level of sunlight intensity. Shading of leaves and bunches, whether natural or artificial, also affected berry composition. It has been shown that the concentrations of anthocyanins and phenolics in grapes are related to the direct effect of light on leaves and to interactions between light and temperature effects on the bunch. Other studies have shown that the phenolic composition of berries may be strongly influenced by cultivar, season, location and viticultural practices, as reviewed by Jackson and Lombard (1993).

The phenolic compounds in the berry skin studied herein included anthocyanins, monomeric flavan-3-ols and proanthocyanidins (tannins) and flavorols (quercetin and quercetin- glycoside). Anthocyanins were the glucosides of malvidin, delphinidin, cyanidin, peonidin and petunidin and their acetyl and coumaroyl derivatives. Monomeric flavan-3-ols included catechin (C), epicatechin (EC), epigallocatechin (EGC) and epicatechin gallate (ECG).
The primary objective of this study was to determine the effect of different light intensity at the bunch zone on the phenolic composition of berry skin and to examine the relationships between phenolic compounds in the berry skin. The approach taken in this study was to compare composition of phenolic compounds at the stage of maximal concentration of anthocyanins (before a decrease at the end of berry ripening (Hrazdina et al. 1984)). The BT treatment was included in this study in the second year in order to separate effects of sunlight at the bunch zone on berries from those effects on leaves.

5.2. Materials and methods
The differences in sunlight intensity at the bunch zone for ST, MET and HET treatment were achieved by canopy manipulation, while elimination of sunlight for BT was obtained by enclosing bunches in specially designed boxes, as described in Chapter 2, section 2.2.2. The temperature at the bunch zone for the BT and HET treatment was monitored during the period from 12\textsuperscript{th} Dec 00 to 26\textsuperscript{th} Dec 00 and from 1\textsuperscript{st} Mar 01 to 5\textsuperscript{th} Mar 01 using thermistor probes, model 6507 A-M and data collected with a 14 channel Macro 32 Starlog data logger, Unidata, Australia.

The concentration and composition of anthocyanins, skin tannins and flavonols was determined on the skin of samples of ST, MET, HET and BT treatments that contained the maximum concentration of total anthocyanins as determined by the UV/visible spectroscopy (as described in Chapter 4). The skins of a 30 berry sample of each replicate (the sample used for the seed tannin analysis) were separated from the berry flesh under liquid nitrogen, cut and separated into two equal lots. One half was used for the analysis of anthocyanins and the other half for the analysis of skin flavan-3-ol monomers and tannins.

Analysis of anthocyanins
The skins were extracted in aqueous 85% acidified ethanol (pH=2) at room temperature for one hour. Following extraction, samples were clarified by centrifugation and filtration (0.45 \(\mu\)m) and diluted (1:2) with distilled water. The HPLC apparatus used was a standard Hewlett-Packard HP1100 instrument equipped with a diode array detector. The column was a 15.0 x 2.0mm Synergy 4u Hydro-RP 80A (Phenomenex) with a guard cartridge packed with the same material. The method utilised an injection volume of 20
μL, flow rate of 0.4 mL/min and a binary gradient with mobile phases containing 1.5 % w/w H₃PO₄ (solvent A) and 20% v/v solvent A in CH₃CN (solvent B). The elution conditions were: 0-18 min solvent A from 85.5 % to 72.5 %, 18-21 min solvent A 72.5%, from 21-22 min solvent A reduced to 45.5%, from 22-26 min solvent A 45.5%, from 26-28 min solvent A 0 % and then the column was washed and re-equilibrated with 82.5% solvent A for 10 min. The separated individual anthocyanin peaks were identified by comparison of their relative retention time. Four different concentrations of a commercial standard of M3G (Extrasynthese, France) were used to construct the calibration curve. One sample of M3G standard was injected after each group of eight analysed samples. The absorbance spectra of each anthocyanin peak was compared with a standard of malvidin-3-glucoside (M3G) and quantified as M3G equivalents.

**Analysis of flavan-3-ol monomers and tannins in the berry skin**

The skins were extracted with aqueous 70% acetone containing 0.05% trifluoroacetic acid (TFA) at room temperature for 24 hours. Following extraction, the extract was concentrated under reduced pressure at 35 °C to remove acetone and residual solution diluted to 25 mL with distilled water. Five mL of that solution was transferred to a sample tube, freeze dried and the solid residue dissolved in 2.5 mL methanol containing 0.05% TFA.

Flavan-3-ol monomers (C, EC, EGC and ECG), flavonols (quercetin and quercetin-3-glucoside) and p-coumaric acid were determined by reverse phase HPLC method (Peng et al. 2002). Prior to analysis samples were clarified by centrifugation at room temperature at 6000 rpm for 10 minutes. The HPLC apparatus used was a standard Hewlett-Packard HP1100 instrument equipped with a diode array detector. The column was 250 x 4.6 mm polystyrene divinylbenzene reverse phase column (PLRP-S 100 A 5 μm, Polymer Laboratories, Church Stretton, Shropshire, UK) with a guard cartridge (10 x 4.6 mm) packed with the same material. The method utilised an injection volume of 20 μL, flow rate of 1 mL/min and a binary gradient with mobile phases containing 1.5% v/v H₃PO₄ (solvent A) and 20% v/v solvent A in CH₃CN (solvent B). The elution conditions were: 0-55 min solvent A from 92 to 73 %, 55-59 min solvent A 73%, from 59-64 min solvent A reduced to 30%, from 64-69 min solvent A 30 %, from 70-76% solvent A increased to 92% and then the column was washed and re-equilibrated with 80% solvent B. Eluting peaks were identified by their retention time and spectral
comparison to known standards of catechin, epicatechin, epicatechin gallate, quercetin, quercetin-3-glucoside and coumaric acid. Catechin, epicatechin, epicatechin gallate and p-coumaric acid standards were purchased from Sigma (Castle Hill, NSW, Australia) and quercetin and quercetin-3-glucoside standards from Extrasynthese (France). Four different concentrations of commercial standards were used to construct the calibration curve. One sample of a standard was injected after each group of eight analysed samples (catechin standard) and after 32 analysed samples (quercetin-3-glucoside standard). The absorbance spectra of each flavan-3-ol monomer peaks was compared with a standard of catechin and quantified as catechin equivalents. Previous studies have found that quercetin-3-glucuronide has similar retention time as quercetin-3-glucoside (Price et al. 1995a, Haselgrove et al. 2000). The employed HPLC method in this study could not resolve quercetin-3-glucuronide from quercetin-3-glucoside, thus the calculated concentrations of quercetin-3-glucoside could be overestimate values.

Tannin concentration, composition and mean degree of polymerisation were determined by acid-catalysis in the presence of excess phloroglucinol (Kennedy and Jones 2001). Prior to HPLC analysis, the methanol extract was diluted (1:1) with a solution containing 1M HCl, 100 g/L phloroglucinol and 20 g/L ascorbic acid in methanol. The mixture was reacted at 50°C for 20 min, and then combined with 5 volumes of 40 mM aqueous sodium acetate. The samples were filtered and analysed by reverse phase HPLC. The column was a Wakosil II 5 C 18 analytical column (250x4.6.mm, particle size 5 mm) purchased from SGE, protected by a guard column containing the same material. The method utilised a binary gradient with mobile phase containing 1% v/v aqueous acetic acid (mobile phase A) and methanol (mobile phase B). Elution conditions were as follows: 1.0 mL/min; 5% B for 10 min, linear gradients from 5 to 20% B in 20 min, and 20 to 40% B in 25 min. The column was washed with 90% B for 10 min and re-equilibrated with 5% B for 5 min before the next injection. Eluting peaks were monitored at 280 nm, identified by their relative retention time and quantified using their absorbance spectra relative to a catechin standard, as described previously in section 4.2.1. The concentration of extension subunit-phloroglucinol adducts was calculated from published molar extinction coefficients (Kennedy and Jones 2001).
5.3. Results
Canopy manipulation for different treatments provided different levels of both leaf and bunch exposure to sunlight. The whole vine shading for the ST treatment reduced sunlight interception at the bunch zone to less than 5% of ambient (<100 photosynthetic photon flux density (PPFD)). Bunches and leaves of the MET treatment were exposed to medium level of sunlight interception of 10-40% of ambient (300-700 PPFD) while bunches and a great proportion of leaves of the HET treatment were exposed to high sunlight intensity of 40-80% of ambient (800-1500 PPFD). Bunches of the BT treatment were enclosed in boxes to eliminate sunlight (>99.5%), while leaves around bunches were highly exposed to sunlight. Light transmission through the box was less than 0.1% between 220 and 800 nm and less than 0.01% below 400nm, while the level of PAR inside the box was less than 0.5% of ambient outside the box (Downey, pers.com). The temperature within the box was similar to the ambient temperature in the canopy (± 0.6°C, data not shown). The temperature of bunches of the ST and MET treatment have not been measured. However, since the experiment was conducted in the warm climate (Barossa Valley), it was assumed that the temperature of highly exposed berries (HET) was higher than that of moderately exposed (MET) and shaded (ST) berries. Ambient temperature was taken daily at 9 am and 3 pm in the vineyard (Appendix 2). During the period of berry ripening (from the beginning of veraison until harvest), 47.5% and 48.5% of daily temperature (in the first and second season respectively) was in the optimal temperature range for anthocyanin biosynthesis (Pirie and Mullins 1977).

5.3.1. Berry weight and concentration of total soluble solids (°Brix)
The average weight of berries developed and ripened without light (BT) was 15% lower than the other treatments (Table 5.1). The difference in the berry weight between the other treatments (ST, MET and HET) was previously described in Chapter 4. The concentration of TSS was not affected by elimination of sunlight and at the same sampling date was even significantly higher (P<0.001) in berries of BT than the other treatments (Table 5.1). This indicated that the accumulation of TSS was entirely due to the leaf exposure to sunlight, as BT treatment had the majority of leaves at the bunch zone highly exposed to sunlight. Slower accumulation of TSS in the ST treatment, reflected as delayed ripening, may be attributed to shading of a great proportion of leaves (as explained previously in Chapter 4).
Table 5.1. The mean berry weight and the concentration of total soluble solids. The berries of the ST, MET, HET and BT treatment were sampled in the 2000 and 2001 season. Each value represents the mean of 8 replicates. Means in columns (± standard error of the mean) followed by different letters are significantly different within one season.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Season</th>
<th>Berry weight (g)</th>
<th>TSS ('Brix)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST</td>
<td>2000</td>
<td>1.34±0.04 a</td>
<td>24.4±0.13</td>
</tr>
<tr>
<td>MET</td>
<td>2000</td>
<td>1.33±0.03 a</td>
<td>24.9±0.16</td>
</tr>
<tr>
<td>HET</td>
<td>2000</td>
<td>1.41±0.03 b</td>
<td>24.9±0.10</td>
</tr>
<tr>
<td>HET</td>
<td>2000</td>
<td>**</td>
<td>ns</td>
</tr>
<tr>
<td>ST</td>
<td>2001</td>
<td>1.07±0.02 b</td>
<td>25.0±0.29 b</td>
</tr>
<tr>
<td>MET</td>
<td>2001</td>
<td>1.12±0.02 b</td>
<td>24.1±0.18 a</td>
</tr>
<tr>
<td>HET</td>
<td>2001</td>
<td>1.08±0.02 b</td>
<td>24.2±0.16 a</td>
</tr>
<tr>
<td>BT</td>
<td>2001</td>
<td>0.92±0.03 a</td>
<td>24.9±0.24 b</td>
</tr>
<tr>
<td>BT</td>
<td>2001</td>
<td>**</td>
<td>***</td>
</tr>
</tbody>
</table>

ns not significant, ***, *** indicate significance at P<0.05, P<0.01, P<0.001 respectively

5.3.2. The concentration and level of anthocyanins in the berry skin

5.3.2.1. The concentration, level and composition of total anthocyanins
Mono-glucoside, acetyl-glucoside and coumaroyl-glucoside derivatives of malvidin, delphinidin, cyanidin, peonidin and petunidin glucoside were determined in the skin of Shiraz berries. In the total anthocyanin pool, malvidin and its derivatives represented more than 70%, peonidin and its derivatives 15%, petunidin and its derivatives 10%, delphinidin and its derivatives 5% and cyanidin and its derivatives only 1%. This composition was not affected by different levels of sunlight intensity at the bunch zone. The maximal concentration (mg/gram berry weight) of total anthocyanins in the skin of berries developed and ripened without sunlight (BT) reached 0.73 which was 50% of that for MET (1.33) and HET (1.38) (Figure 5.1). A small amount of sunlight interception (less than 5% of ambient) for ST decreased the concentration to 75-80% (0.97 mg) of that for MET and HET (Figure 5.1). The maximal level (mg/berry) of total anthocyanins for different treatments was as follows: 1.60 and 1.04 (ST), 2.01 and 1.49 (MET), 2.01 and 1.49 (HET) in the first and second season respectively and 0.67 (BT) in the second season. There was no significant difference (P<0.05) in the concentration and level of anthocyanins between MET and HET.
Figure 5.1. The concentration and level of (a) total anthocyanins, (b) anthocyanins in the monoglucoside form, (c) anthocyanins in the acetyl-glucoside form and (d) anthocyanins in the coumaroyl-glucoside form in the skins of berries developed and ripened under different light conditions. The berries of the following treatments: # BT (0% sunlight at the bunch zone), ■ ST (1-10% sunlight at the bunch zone), ● MET (10-50% sunlight at the bunch zone) and □ HET (40-80% sunlight at the bunch zone) were sampled in the 2000 and 2001 season. Error bars represent ± standard error of the mean (n=8). Different letters above error bars indicate significant difference (P<0.05) between means within one season. Note the different y-axis scales.
The concentration and level of anthocyanins in the mono-glucoside form were the most affected by different light intensity and both were reduced by 60% for BT and 30% and 45% for ST (in the first and second season respectively) when compared to MET and HET. The concentration and level of anthocyanins in the acetyl-glucoside form was significantly ($P<0.001$) lower: 50% for BT and 15 and 20% for ST (in the first and second season respectively) while total anthocyanins in the coumaroyl-glucoside form was reduced by 30% for BT and 9% and 17% for ST in the first and second season respectively (Figure 5.1). Different light conditions for the MET and HET treatment did not affect the concentration and level of anthocyanins in the mono-glucoside form, acetyl-glucoside form and coumaroyl-glucoside form.

Excluded light (BT) or low light interception (ST) significantly ($P<0.001$) reduced the proportion of anthocyanins in the mono-glucoside form but increased the proportion of anthocyanins in the coumaroyl-glucoside form, when compared to MET and HET (Table 5.2). The least affected was the proportion of anthocyanins in the acetyl-glucoside form (Table 5.2).

Table 5.2. Proportions of anthocyanins in the mono-glucoside form, acetyl-glucoside form and coumaroyl-glucoside form in the skins of berries developed and ripened under different light conditions. The berries of the ST, MET, HET and BT treatment were sampled in the 2000 and 2001 season. Each value represents the mean of 8 replicates. Means in columns followed by different letters are significantly different within one season.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Year</th>
<th>Total mono-glucoside (%)</th>
<th>Total acetyl-glucoside (%)</th>
<th>Total coumaroyl glucoside (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST</td>
<td>2000</td>
<td>36.8 a</td>
<td>20.7</td>
<td>42.6 b</td>
</tr>
<tr>
<td>MET</td>
<td>2000</td>
<td>42.2 b</td>
<td>19.7</td>
<td>38.1 a</td>
</tr>
<tr>
<td>HET</td>
<td>2000</td>
<td>43.3 b</td>
<td>21.1</td>
<td>37.4 a</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>***</td>
<td>ns</td>
<td>***</td>
</tr>
<tr>
<td>ST</td>
<td>2001</td>
<td>28.7 a</td>
<td>22.3 b</td>
<td>49.1 b</td>
</tr>
<tr>
<td>MET</td>
<td>2001</td>
<td>36.7 b</td>
<td>20.6 ab</td>
<td>42.8 a</td>
</tr>
<tr>
<td>HET</td>
<td>2001</td>
<td>35.9 b</td>
<td>20.8 ab</td>
<td>43.3 a</td>
</tr>
<tr>
<td>BT</td>
<td>2001</td>
<td>27.1 a</td>
<td>18.4 a</td>
<td>54.5 c</td>
</tr>
<tr>
<td></td>
<td>2001</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

ns not significant, *, **, *** indicate significance at $P<0.05$, $P<0.01$, $P<0.001$ respectively
Differences between treatments and seasons

When compared between seasons, the maximal concentration of total anthocyanins in the second season was reduced by 21% for ST, 12% for MET and only 3% for HET (Figure 5.1). The maximal level of anthocyanins was lower by 36%, 26% and 26% (ST, MET and HET respectively) which was due to both lower accumulation and lower berry size in the second season (Figure 5.1). The proportion of different forms of anthocyanins differed significantly ($P<0.001$) between seasons with a higher proportion of anthocyanins in the mono-glucoside form (37-43%) and lower proportion of anthocyanins in the coumaroyl form (37-43%) in the first season, while the proportion of anthocyanins in the acetyl-glucoside form was similar for both seasons (20-22%) (Table 5.2).

5.3.2.2. The concentration and proportion of individual anthocyanins in the mono-glucoside form

The concentration (mg/gram berry weight) of individual anthocyanins in the mono-glucoside form was significantly ($P<0.001$) reduced in the skin of berries developed and ripened without light (BT) or under low sunlight interception (ST) (Figure 5.2). In the berry skin of BT the accumulation of mono-glucoside derivatives of delphinidin was reduced by 80%, petunidin 65%, malvidin 60%, cyanidin 55% and peonidin 50%, when compared to MET and HET (Figure 5.2). Under low sunlight conditions (ST) the concentrations of delphinidin and petunidin were reduced by 30% and 60% (in the first and second season respectively), cyanidin 40%, peonidin 30% and malvidin 30 and 40% (in the first and second season respectively) when compared to MET and HET (Figure 5.2). The concentration of individual anthocyanins in mono glucoside form did not differ significantly ($P<0.05$) in the berry skin of MET and HET (Figure 5.2).
Figure 5.2. The concentration of individual anthocyanins in the mono-glucoside form: (a) delphinidin mono-glucoside, (b) cyanidin mono-glucoside, (c) petunidin mono-glucoside, (d) peonidin mono-glucoside and (e) malvidin mono-glucoside in the coumaroyl-glucoside form in the skins of berries developed and ripened under different light conditions. The berries of the following treatments: # BT (0% sunlight at the bunch zone), ■ ST (1-10% sunlight at the bunch zone), ⊘ MET (10-50% sunlight at the bunch zone) and □ HET (40-80% sunlight at the bunch zone) were sampled in the 2000 and 2001 season. Error bars represent ± standard error of the mean (n=8). Different letters above error bars indicate significant difference (P<0.05) between means within one season. Note the different y-axis scales.
While malvidin mono-glucoside represented 29% and 27% of total anthocyanins in the berry skin of MET and HET, in the skin of ST it represented 26% and 22% (in the first and second season respectively) and only 21% for BT (Table 5.3). Reduced light conditions decreased the proportion of delphinidin and petunidin mono-glucoside in both ST and BT, while the proportions of cyanidin and peonidin were reduced only in the first season (Table 5.3). Different percentage of sunlight received at the bunch zone for MET and HET treatments did not significantly affect the proportion of individual anthocyanins in the mono-glucoside form, except for cyanidin mono-glucoside in the first season (Table 5.3).

Table 5.3. Proportions of individual anthocyanins in the mono-glucoside form in the skins of berries developed and ripened under different light conditions. The berries of the ST, MET, HET and BT treatment were sampled in the 2000 and 2001 season. Each value represents the mean of 8 replicates. Means in columns followed by different letters are significantly different within one season.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Year</th>
<th>Delphinidin mono-glucoside (%)</th>
<th>Cyanidin mono-glucoside (%)</th>
<th>Petunidin mono-glucoside (%)</th>
<th>Peonidin mono-glucoside (%)</th>
<th>Malvidin mono-glucoside (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST</td>
<td>2000</td>
<td>2.2 a</td>
<td>0.3 a</td>
<td>3.4 a</td>
<td>5.0 a</td>
<td>25.9 a</td>
</tr>
<tr>
<td>MET</td>
<td>2000</td>
<td>2.7 b</td>
<td>0.4 b</td>
<td>4.1 b</td>
<td>6.0 ab</td>
<td>29.1 b</td>
</tr>
<tr>
<td>HET</td>
<td>2000</td>
<td>2.8 b</td>
<td>0.5 c</td>
<td>4.4 b</td>
<td>6.5 b</td>
<td>29.0 b</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>**</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>ST</td>
<td>2001</td>
<td>1.2 a</td>
<td>0.3</td>
<td>1.8 a</td>
<td>3.8 b</td>
<td>21.6 a</td>
</tr>
<tr>
<td>MET</td>
<td>2001</td>
<td>2.2 b</td>
<td>0.3</td>
<td>3.2 b</td>
<td>3.7 b</td>
<td>27.4 b</td>
</tr>
<tr>
<td>HET</td>
<td>2001</td>
<td>2.0 b</td>
<td>0.3</td>
<td>2.9 b</td>
<td>3.9 b</td>
<td>26.8 b</td>
</tr>
<tr>
<td>BT</td>
<td>2001</td>
<td>0.8 a</td>
<td>0.3</td>
<td>1.9 a</td>
<td>3.4 a</td>
<td>21.0 a</td>
</tr>
<tr>
<td></td>
<td>2001</td>
<td>***</td>
<td>ns</td>
<td>***</td>
<td>**</td>
<td>***</td>
</tr>
</tbody>
</table>

** as not significant, ***,*** indicate significance at P<0.05, P<0.01, P<0.001 respectively

5.3.2.3. The concentration and proportion of individual anthocyanins in the acetyl-glucoside form

In the skins of berries developed and ripened without light (BT) the concentration of acetyl-glucoside derivatives of petunidin was reduced by 70%, delphinidin 60%, malvidin 50%, peonidin 40% and cyanidin 40-50% compared to MET and HET. When berries ripened under a small level of sunlight interception (less than 5% of ambient)
(ST) the concentration of individual anthocyanins in the acetyl-glucoside form was greater than in BT, but compared to MET and HET, the concentration of delphinidin was reduced by 45% and 30%, petunidin 25% and 40% cyanidin 30% and 10%, malvidin 10% and 25% (in the first and second season respectively), while peonidin acetyl-glucoside was not significantly affected (P<0.05) (Figure 5.3).

Figure 5.3. The concentration of individual anthocyanins in the acetyl-glucoside form: (a) delphinidin acetyl-glucoside, (b) cyanidin acetyl-glucoside, (c) petunidin acetyl-glucoside, (d) peonidin acetyl-glucoside and (e) malvidin acetyl-glucoside in the skin of berries developed and ripened under different light conditions. The berries of the following treatments: # BT (0 % sunlight at the bunch zone), ■ ST (1-10% sunlight at the bunch zone), □ MET (10-50% sunlight at the bunch zone) and ▲ HET (40-80% sunlight at the bunch zone) were sampled in the 2000 and 2001 season. Error bars represent ± standard error of the mean (n=8). Different letters above error bars indicate significant difference (P<0.05) between means within one season. Note the different y-axis scales.
When comparing MET and HET the concentrations of individual anthocyanins in the acetyl-glucoside form was similar except for cyanidin acetyl-glucoside which was 35% higher for HET in the second season and peonidin acetyl-glucoside which was 16% higher for MET in the first season (Figure 5.3).

Excluded sunlight (BT) or reduced sunlight interception (ST) significantly \( (P<0.001) \) decreased the proportion of petunidin acetyl-glucoside and increased the proportion of peonidin acetyl-glucoside when compared to MET and HET (Table 5.4). The proportion of malvidin acetyl-glucoside was significantly \( (P<0.001) \) reduced only for BT. Proportions of delphinidin and cyanidin acetyl-glucoside varied between treatments and seasons. There were no significant difference between the proportion of individual anthocyanins in the acetyl-glucoside form between MET and HET (Table 5.4).

Table 5.4. Proportions of individual anthocyanins in the acetyl-glucoside form in the skins of berries developed and ripened under different light conditions. The berries of the ST, MET, HET and BT treatment were sampled in the 2000 and 2001 season. Each value represents the mean of 8 replicates. Means in columns followed by different letters are significantly different within one season.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Year</th>
<th>Delphinidin-acetyl-glucoside (%)</th>
<th>Cyanidin-acetyl-glucoside (%)</th>
<th>Petunidin-acetyl-glucoside (%)</th>
<th>Peonidin-acetyl-glucoside (%)</th>
<th>Malvidin-acetyl-glucoside (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST</td>
<td>2000</td>
<td>0.5 a</td>
<td>0.2</td>
<td>1.2 a</td>
<td>3.6 b</td>
<td>15.1</td>
</tr>
<tr>
<td>MET</td>
<td>2000</td>
<td>0.8 b</td>
<td>0.2</td>
<td>1.5 b</td>
<td>3.0 a</td>
<td>14.2</td>
</tr>
<tr>
<td>HET</td>
<td>2000</td>
<td>0.8 b</td>
<td>0.2</td>
<td>1.5 b</td>
<td>2.7 a</td>
<td>14.1</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>**</td>
<td>ns</td>
<td>**</td>
<td>***</td>
<td>ns</td>
</tr>
<tr>
<td>ST</td>
<td>2001</td>
<td>0.5 b</td>
<td>0.3 b</td>
<td>0.9 b</td>
<td>4.0 b</td>
<td>16.6 b</td>
</tr>
<tr>
<td>MET</td>
<td>2001</td>
<td>0.5 b</td>
<td>0.2 a</td>
<td>1.1 c</td>
<td>3.0 a</td>
<td>15.8 b</td>
</tr>
<tr>
<td>HET</td>
<td>2001</td>
<td>0.5 b</td>
<td>0.2 a</td>
<td>1.1 c</td>
<td>3.0 a</td>
<td>15.9 b</td>
</tr>
<tr>
<td>BT</td>
<td>2001</td>
<td>0.4 a</td>
<td>0.2 a</td>
<td>0.6 a</td>
<td>3.3 a</td>
<td>13.9 a</td>
</tr>
<tr>
<td></td>
<td>2001</td>
<td>**</td>
<td>*</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

\textit{ns} not significant, *, **, *** indicate significance at \( P<0.05, P<0.01, P<0.001 \) respectively
5.3.2.4. The concentration and proportion of individual anthocyanins in the coumaroyl-glucoside form

Complete elimination of sunlight at the bunch significantly (P<0.001) affected the concentration of individual anthocyanins in the coumaroyl-glucoside form and delphinidin was reduced by 60%, petunidin 60%, cyanidin 50%, malvidin 30% and peonidin only 15% (Figure 5.4).

Figure 5.4. The concentration of individual anthocyanins in the coumaroyl-glucoside form: (a) delphinidin coumaroyl-glucoside, (b) cyanidin coumaroyl-glucoside, (c) petunidin coumaroyl-glucoside, (d) peonidin coumaroyl-glucoside and (e) malvidin coumaroyl-glucoside in the skin of berries developed and ripened under different light conditions. The berries of the following treatments: # BT (0 % sunlight at the bunch zone), ■ ST (1-10% sunlight at the bunch zone), ■ MET (10-50% sunlight at the bunch zone) and □ HET (40-80% sunlight at the bunch zone) were sampled in the 2000 and 2001 season. Error bars represent ± standard error of the mean (n=8). Different letters above error bars indicate significant difference (P<0.05) between means within one season. Note the different y-axis scales.
When berries were grown under reduced sunlight (less than 5% of ambient) the concentration of delphinidin was reduced by 25% and 40%, petunicin 16% and 35% and malvidin coumaroyl-glucoside by 10% and 18% compared to MET and HET (in the first and second season respectively). The concentrations of cyanidin and peonidin coumaroyl-glucoside were similar for ST, MET and HET in both seasons except the concentration of cyanidin coumaroyl-glucoside for ST in the first season (Figure 5.4). When comparing MET and HET the concentrations of individual anthocyanins in the coumaroyl-glucoside form were similar except for the concentration of malvidin coumaroyl-glucoside in the first season (Figure 5.4).

Table 5.5. Proportions of individual anthocyanins in the coumaroyl-glucoside form in the skins of berries developed and ripened under different light conditions. The berries of the ST, MET, HET and BT treatment were sampled in the 2000 and 2001 season. Each value represents the mean of 8 replicates. Means in columns followed by different letters are significantly different within one season.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Year</th>
<th>Delphinidin coumaroyl-glucoside (%)</th>
<th>Cyanidin coumaroyl-glucoside (%)</th>
<th>Petunidin coumaroyl-glucoside (%)</th>
<th>Peonidin coumaroyl-glucoside (%)</th>
<th>Malvidin coumaroyl-glucoside (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST</td>
<td>2000</td>
<td>2.2 a</td>
<td>0.7 b</td>
<td>3.9</td>
<td>8.4 b</td>
<td>27.3 b</td>
</tr>
<tr>
<td>MET</td>
<td>2000</td>
<td>2.4 ab</td>
<td>0.5 a</td>
<td>3.9</td>
<td>7.0 a</td>
<td>24.4 a</td>
</tr>
<tr>
<td>HET</td>
<td>2000</td>
<td>2.6 b</td>
<td>0.5 a</td>
<td>4.0</td>
<td>6.8 a</td>
<td>23.6 a</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>**</td>
<td>***</td>
<td>ns</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>ST</td>
<td>2001</td>
<td>1.7 a</td>
<td>0.9 b</td>
<td>3.5 a</td>
<td>8.5 b</td>
<td>34.4 b</td>
</tr>
<tr>
<td>MET</td>
<td>2001</td>
<td>1.9 b</td>
<td>0.6 a</td>
<td>3.9 b</td>
<td>6.6 a</td>
<td>29.8 a</td>
</tr>
<tr>
<td>HET</td>
<td>2001</td>
<td>2.1 c</td>
<td>0.6 a</td>
<td>3.9 b</td>
<td>6.6 a</td>
<td>30.4 a</td>
</tr>
<tr>
<td>BT</td>
<td>2001</td>
<td>1.4 a</td>
<td>0.6 a</td>
<td>3.0 a</td>
<td>10.2 b</td>
<td>39.1 c</td>
</tr>
<tr>
<td></td>
<td>2001</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

ns not significant, **,**,** indicate significance at P<0.05, P<0.01, P<0.001 respectively

Excluded (BT) or reduced sunlight interception at the bunch zone (ST) increased significantly (P<0.001) the proportion of cyanidin, peonidin and malvidin coumaroyl-glucoside and decreased the proportion of delphinidin coumaroyl-glucoside in total
The proportion of petunidin coumaroyl-glucoside was not affected in the first season, but was reduced for ST in the second season. MET and HET had similar proportions of all individual anthocyanins in the coumaroyl-glucoside form, except delphinidin coumaroyl-glucoside (Table 5.5).

5.3.2.5. Relationships between the concentration and level of anthocyanins and sunlight interception at the bunch zone (PAR)

The concentrations (mg/gram berry weight) and levels (mg/berry) of total and individual anthocyanins were plotted against sunlight intensity at the bunch zone (PAR) (Figure 5.5.a and Figure 5.5.b). Each relationship was found to follow a positive polynomial relationship. Sunlight intensity at the bunch zone strongly correlated with the concentration (mg/gram berry weight) and level (mg/berry) of anthocyanins in the acylated and non-acylated forms in the following order: anthocyanins in mono-glucoside form > total anthocyanins > anthocyanins in the acetyl-glucoside form > anthocyanins in the coumaroyl-glucoside form (Figure 5.5.a and Figure 5.5.b). A comparison of fitted curves between the concentration and level of anthocyanins at the same season showed similar slopes of the curves, but the value of the coefficient of determination was slightly higher in relation to per berry than per gram berry weight.

In both seasons the relationship between the concentration and level of total anthocyanins in the mono-glucoside form and sunlight intensity at the bunch zone was defined as a highly significant \( (P<0.001) \) polynomial relationship of the second degree. The fitted curve for glucosides showed a maximum at around 50% of ambient (800 PPFD) (Figure 5.5.a and Figure 5.5.b). The relationship between acylated anthocyanins and sunlight intensity at the bunch zone was best fitted by a polynomial equation of the second degree with a maximum around 50% of ambient (800 PPFD) (Figure 5.5.a and Figure 5.5.b). The coefficient of determination \( (r^2) \) indicated a strong relationship between variables, particularly in the second season. The coefficient of determination \( (r^2) \) of the relationship between the concentration and level of anthocyanins in the coumaroyl-glucoside form and sunlight at the bunch zone indicated less strong relationship between variables than for the glucosides and acetates (Figure 5.5a and Figure 5.5b). The fitted curve for coumarates showed a maximum of 50-60% of ambient (800-1000 PPFD) (Figure 5.5.a and Figure 5.5.b).
Figure 5.5.a. The concentration of total anthocyanins, anthocyanins in the mono-glucoside form, anthocyanins in the acetyl-glucoside form and anthocyanins in the coumaroyl-glucoside form in relation to the sunlight interception at the bunch zone. The berries of the following treatments: ○ BT (0 % sunlight at the bunch zone), ● ST (1-10% sunlight at the bunch zone), ■ MET (10-50% sunlight at the bunch zone) and △ HET (40-80% sunlight at the bunch zone) were sampled in (a) 2000 and (b) 2001 season. Significance of the coefficient of determination is determined by: *, **, *** significant at P<0.05, P<0.01 and P<0.001.
Figure 5.5.b. The level of total anthocyanins, anthocyanins in the mono-glucoside form, anthocyanins in the acetyl-glucoside form and anthocyanins in the coumaroyl-glucoside form in relation to the sunlight interception at the bunch zone. The berries of the following treatments: ○ BT (0 % sunlight at the bunch zone), • ST (1-10% sunlight at the bunch zone), ▲ MET (10-50% sunlight at the bunch zone) and △ HET (40-80% sunlight at the bunch zone) were sampled in (a) 2000 and (b) 2001 season. Significance of the coefficient of determination is determined by: *, **, *** significant at P<0.05, P<0.01 and P<0.001.
The relationships between the concentration of individual anthocyanins and their derivatives and sunlight interception at the bunch zone were best fitted as polynomial relationships of the second degree (data not shown). All relationships were statistically significant at $P<0.001$ (malvidin, delphinidin and petunidin in both seasons, cyanidin and peonidin in the second season) and $P<0.05$ (cyanidin and peonidin in the first season). The accumulation of the major anthocyanins constituent, malvidin and its derivatives was strongly correlated to sunlight interception and the coefficient of determination ($r^2$) was 0.69 and 0.81 in the first and second season respectively. Strong correlations were established for petunidin ($r^2=0.71$ and 0.88) and delphinidin ($r^2=0.69$ and 0.86 in the first and second season respectively), while for both cyanidin and peonidin and their derivatives the coefficient of determination ($r^2$) was 0.30 and 0.64 (in the first and second season respectively).

5.3.3. The concentration, level and composition of monomeric flavan-3-ols and tannins in the skin
Monomeric flavan-3-ols (catechin (C), epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC)) and tannins (polymeric compounds composed of flavan-3-ol terminal and extension subunits) were determined in the berry skin of Shiraz grapes to investigate the effects of different levels of sunlight interception on the concentration and composition of flavan-3-ol monomers and tannins in the berry skin.

5.3.3.1. The concentration and level of monomers and tannins in the berry skin
The concentration (mg/gram berry weight) of total tannins in the berry skin of Shiraz grapes increased with increasing sunlight interception at the bunch zone (Figure 5.6). The complete exclusion of sunlight significantly reduced ($P<0.001$) the accumulation of tannins in the berry skin of BT (0.38) by 15%, 30% and 40% when compared to other treatments (ST, MET and HET respectively). When berries developed and ripened under low light conditions (ST) (0.41 and 0.44) the concentration of total tannins was reduced by 30 and 20% and 40% and 30% compared to MET (0.58 and 0.56) and HET (0.64 and 0.63) (in the first and second season respectively) (Figure 5.6). Higher sunlight interception for HET (40 100% of ambient) increased the concentration of total tannins by 10% when compared to MET (10-40% of ambient).
Figure 5.6. The concentration and level of (a) total tannins, (b) extension subunits, (c) terminal subunits and (d) monomer flavan-3-ols in the skin of berries developed and ripened under different light conditions. The berries of the following treatments: # BT (0 % sunlight at the bunch zone), ■ ST (1-10% sunlight at the bunch zone), ≈ MET (10-50% sunlight at the bunch zone) and □ HET (40-80% sunlight at the bunch zone) were sampled in the 2000 and 2001 season. Error bars represent ± standard error of the mean (n=8). Different letters above error bars indicate significant difference (P<0.05) between means within one season. Note the different y-axis scales.
The extension subunits represented more than 90% of total tannins in the berry skin, and the same trend of increased concentration with increased sunlight interception was observed (Figure 5.6). No major difference was observed in the concentration of terminal subunits and monomer flavan-3-ols when berries developed and ripened without light (BT) or a little light (ST), but compared to berries grown under greater sunlight interception (MET and HET), the accumulation of terminal subunits was reduced by 20-25% and monomer flavan-3-ols by 30-40% (Figure 5.6). Although there was a great reduction in the concentration of terminal subunits and monomers, it should be noted that they represented less than 10% of the total tannin amount.

The level (mg/berry) of total tannins and respective subunits in the berry skin of different treatments followed the same increasing trend as that of the concentration of skin tannins (Figure 5.6).

Different levels of sunlight interception at the bunch zone had only a little effect on the composition of constitutive extension and terminal subunits and monomer flavan-3-ols (Table 5.6). The composition of extension subunits was primarily epicatechin (48-57%) and epigallocatechin (33-45%) while epicatechin gallate represented only 7-8% and catechin 2-3%. The most abundant in the terminal subunits was catechin (47-70%), then epicatechin (24-50%) and epigallocatechin (5-13%). The composition of monomers was predominantly epigallocatechin (33-41%) and epicatechin gallate (25-43%), while catechin (8-18%) and epicatechin (12-21%) remained relatively low (Table 5.6).
Table 5.6. Proportions of catechin (C), epicatechin (EC), epigallocatechin (EGC) and epicatechin gallate (ECG) in the extension and terminal subunits and monomers in the skins of berries developed and ripened under different light conditions. The berries of the ST, MET, HET and BT treatment were sampled in the 2000 and 2001 season. Each value represents the mean of 8 replicates. Means in columns followed by different letters are significantly different within one season.

<table>
<thead>
<tr>
<th>Treat.</th>
<th>Year</th>
<th>Extension subunits</th>
<th>C</th>
<th>EC</th>
<th>EGC</th>
<th>ECG</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST</td>
<td>2000</td>
<td>3.1 b</td>
<td>50.6 b</td>
<td>39.3 a</td>
<td>7.1</td>
<td></td>
</tr>
<tr>
<td>MET</td>
<td>2000</td>
<td>2.0 a</td>
<td>44.8 a</td>
<td>45.2 b</td>
<td>7.9</td>
<td></td>
</tr>
<tr>
<td>HET</td>
<td>2000</td>
<td>1.5 a</td>
<td>46.5 a</td>
<td>44.7 b</td>
<td>7.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>ST</td>
<td>2001</td>
<td>2.9 a</td>
<td>57.0 b</td>
<td>32.5 a</td>
<td>7.7 a</td>
<td></td>
</tr>
<tr>
<td>MET</td>
<td>2001</td>
<td>2.6 a</td>
<td>52.6 a</td>
<td>37.5 b</td>
<td>7.3 a</td>
<td></td>
</tr>
<tr>
<td>HET</td>
<td>2001</td>
<td>2.4 a</td>
<td>53.3 a</td>
<td>35.2 ab</td>
<td>7.1 a</td>
<td></td>
</tr>
<tr>
<td>BT</td>
<td>2001</td>
<td>3.3 b</td>
<td>55.9 ab</td>
<td>30.7 a</td>
<td>10.2 b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2001</td>
<td>** **</td>
<td>*</td>
<td>*</td>
<td>***</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Terminal subunits</th>
<th>C</th>
<th>EC</th>
<th>EGC</th>
<th>ECG</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST 2000</td>
<td>51.0 a</td>
<td>38.9 b</td>
<td>10.1 b</td>
<td></td>
</tr>
<tr>
<td>MET 2000</td>
<td>59.4 a</td>
<td>35.5 b</td>
<td>5.1 a</td>
<td></td>
</tr>
<tr>
<td>HET 2000</td>
<td>70.6 b</td>
<td>24.3 a</td>
<td>5.1 a</td>
<td></td>
</tr>
<tr>
<td>ST 2001</td>
<td>46.4</td>
<td>45.8</td>
<td>7.8 a</td>
<td></td>
</tr>
<tr>
<td>MET 2001</td>
<td>47.7</td>
<td>39.0</td>
<td>13.4 b</td>
<td></td>
</tr>
<tr>
<td>HET 2001</td>
<td>50.0</td>
<td>43.1</td>
<td>7.4 a</td>
<td></td>
</tr>
<tr>
<td>BT 2001</td>
<td>45.2</td>
<td>45.2</td>
<td>13.6 b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ns</td>
<td>ns</td>
<td>***</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Flavan-3-ol monomers</th>
<th>C</th>
<th>EC</th>
<th>EGC</th>
<th>ECG</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST 2000</td>
<td>16.0 b</td>
<td>18.5 b</td>
<td>39.2</td>
<td>26.4 a</td>
</tr>
<tr>
<td>MET 2000</td>
<td>15.3 b</td>
<td>11.6 a</td>
<td>40.6</td>
<td>32.6 b</td>
</tr>
<tr>
<td>HET 2000</td>
<td>8.0 a</td>
<td>13.6 a</td>
<td>35.1</td>
<td>43.3 c</td>
</tr>
<tr>
<td>ST 2001</td>
<td>18.3 b</td>
<td>15.6 a</td>
<td>33.5</td>
<td>32.6 b</td>
</tr>
<tr>
<td>MET 2001</td>
<td>14.2 a</td>
<td>20.4 b</td>
<td>40.4</td>
<td>25.0 a</td>
</tr>
<tr>
<td>HET 2001</td>
<td>11.2 a</td>
<td>20.8 b</td>
<td>36.8</td>
<td>31.2 b</td>
</tr>
<tr>
<td>BT 2001</td>
<td>17.2 b</td>
<td>28.9 c</td>
<td>37.3</td>
<td>16.7 a</td>
</tr>
<tr>
<td></td>
<td>ns</td>
<td>ns</td>
<td>***</td>
<td></td>
</tr>
</tbody>
</table>

ns not significant, **,**,** indicate significance at $P<0.05$, $P<0.01$, $P<0.001$ respectively

**Polymer size (mDP)**

Polymer size of tannins or mean degree of polymerisation was significantly lower ($P<0.001$) in the skin of berries grown without light (19.6 for BT) or under a low light (25.6 and 21.9 for ST) when compared to berries grown under moderate (27.4 and 24.1 for MET) or high intensity of light (27.0 and 24.3 for HET in the first and second season respectively).

**Difference between seasons**

The concentration of total tannins in the berry skin was similar in both seasons, but a difference in the concentration of monomeric flavan-3-ols and the concentration of terminal subunits between seasons and treatments was observed. In the second season in the berry skin of ST the concentration of monomers increased by 30%, but the concentration of terminal subunits decreased 20%. MET had an increase for 3% and 9%, and HET 20% and 10% (for monomers and terminal units respectively). In the
second season polymer size (mDP) decreased only for ST (15%) due to lower concentration of terminal subunits in the second season, as the concentration of extension subunits was similar in both seasons. The level of monomers and tannins showed greater differences between seasons due to the difference in the berry size.

5.3.3.2. Relationship between the concentration and level of tannins in the berry skin and sunlight interception at the bunch zone (PAR)

The relationship between the concentration and level of total tannins in the berry skin and sunlight intensity at the bunch zone was defined as a highly significant (P<0.001) polynomial relation of second degree. The fitted curve showed a maximum at 60-70% of ambient (1000-1200 PPFD) (Figure 5.7).

![Figure 5.7.](image_url)

Figure 5.7. The concentration and level of total skin tannins in relation to sunlight interception at the bunch zone. The berries of the following treatments: o BT (0 % sunlight at the bunch zone), ST (1-10% sunlight at the bunch zone), MET (10-50% sunlight at the bunch zone) and A HET (40-80% sunlight at the bunch zone) were sampled in (a) 2000 and (b) 2001 season. Significance of the coefficient of determination is determined by: *, **, *** significant at P<0.05, P<0.01 and P<0.001.
5.3.4. The concentration and level of quercetin-3-glucoside and quercetin in the berry skin

5.3.4.1 The concentration and level of quercetin-3-glucoside and quercetin

The concentration (mg/g berry weight) and level (mg/berry) of quercetin-3-glucoside increased with increasing sunlight interception. The concentration of quercetin-3-glucoside was as follows: 0.025 and 0.021 (ST), 0.133 and 0.048 (MET), 0.161 and 0.057 (HET) and 0 mg/g berry weight (BT) in the first and second season respectively. The concentration of quercetin was as follows: 0.0003 and 0.0002 (ST), 0.0016 and 0.0010 (MET), 0.0020 and 0.0015 (HET) and 0.0001 mg/g berry weight (BT) in the first and second season respectively. The data showed that the accumulation of quercetin-3-glucoside was completely inhibited in the skin of berries grown without light (BT), while the accumulation of quercetin was reduced by 40, 90 and 95% compared to ST, MET and HET respectively. Reduced light conditions significantly (P<0.001) decreased the concentration and level of quercetin-3-glucoside in the berry skin of ST by 80% and 85% and 60 and 65% when compared to MET and HET (in the first and second season respectively) (Figure 5.8). A comparison between MET and HET showed significantly (P<0.001) higher concentration and level of quercetin-3-glucoside in the skin of HET (20%) (Figure 5.8).
Figure 5.8. The concentration and level of quercetin-3-glucoside and quercetin in the skin of berries developed and ripened under different light conditions. The berries of the following treatments: # BT (0 % sunlight at the bunch zone), ■ ST (1-10% sunlight at the bunch zone), □ MET (10-50% sunlight at the bunch zone) and ▲ HET (40-80% sunlight at the bunch zone) were sampled in the 2000 and 2001 season. Error bars represent ± standard error of the mean (n=8). Different letters above error bars indicate significant difference (P<0.05) between means within one season. Note the different y-axis scales.

5.3.4.2. Relationships between the concentration and level of quercetin-3-glucoside and quercetin in the berry skin and sunlight interception at the bunch zone (PAR)

The relationships between the concentration and level of quercetin-3-glucoside and quercetin and sunlight interception at the bunch zone were defined as highly significant (P<0.001) polynomial relationships of the second degree (Figure 5.9). The fitted curve showed a maximum around 50-60% of ambient (800-1000 PPFD).
Figure 5.9. The concentration and level of quercetin-3-glucoside and quercetin in the berry skin in relation to sunlight interception at the bunch zone. The berries of the following treatments: ○ BT (0% sunlight at the bunch zone), ⋆ ST (1-10% sunlight at the bunch zone), ■ MET (10-50% sunlight at the bunch zone) and △ HET (40-80% sunlight at the bunch zone) were sampled in (a) 2000 and (b) 2001 season. Significance of the coefficient of determination is determined by: *, **, *** significant at $P<0.05$, $P<0.01$ and $P<0.001$. 
The accumulation of quercetin-3-glucoside and quercetin significantly increased with the increase of sunlight intensity (Figure 5.8). The relationship between the concentration of both quercetin-3-glucoside and quercetin, and sunlight intensity at the bunch zone was found to be a highly significant ($P<0.001$) polynomial relationship of the second degree with the coefficient of determination ($r^2$) of 0.82-0.91 for quercetin-3-glucoside and 0.88-0.93 for quercetin (Figure 5.9). It should be noted that although fitted polynomial curves showed slightly downward trends at higher values of sunlight intensity, generally it may be expected that accumulation of quercetin-3-glucoside and quercetin increase with increasing levels of sunlight intensity.

5.3.5. Relationships between phenolic compounds in the berry
The relationships between i) the concentration of total anthocyanins and skin tannins and ii) the concentration of skin tannins and total flavonols (sum of quercetin and quercetin-3-glucoside) were found to be linear relationships. The analysis of variance of the linear regression showed a significant relationship between concentration of skin tannins and the concentration anthocyanins (the coefficient of determination $r^2$ was 0.32, $P<0.01$ and 0.64, $P<0.001$) and between the concentration of skin tannins and the concentration of flavonols ($r^2$ was 0.69 and 0.64, $P<0.001$, in the first and second season respectively).

![Figure 5.10](image-url)

Figure 5.10. The relationships between the concentration of skin tannins and (a) the concentration of total anthocyanins and (b) the concentration of flavonols in 2000 and 2001 season. Solid lines represent the linear relationship between variables in each season. Significance of the correlation coefficient is determined by **, *** significant at $P<0.01$ and $P<0.001$. 
It should be noted that the significance of both correlations could be driven from large number of samples, as there was a wide spread of points on both sides of a trendline (Figure 5.10).

The relationships between different forms of anthocyanins (glucosides, acetates and coumarates) and skin tannins have also been examined. In the relationships between anthocyanins and skin tannins the values of the correlation coefficient \( r \) were as follows: 0.72 \((P<0.001)\) and 0.80 \((P<0.001)\) for glucosides, 0.40 \((P<0.05)\) and 0.78 \((P<0.001)\) for acetates, and 0.18 and 0.76 \((P<0.001)\) for coumarates. In the relationship with total anthocyanins the correlation coefficient \( r \) had similar values for both quercetin (0.69 and 0.62, \( P<0.001 \)) and quercetin-3-glucoside (0.86 and 0.89, \( P<0.001 \)). In the relationship with skin tannins the correlation coefficient \( r \) was 0.74 and 0.82 \((P<0.001)\) for quercetin and 0.83 and 0.75 \((P<0.001)\) for quercetin-3-glucoside (in the first and second season respectively).

5.4. Discussion

The primary objective of this study was to determine the content and composition of phenolic compounds in the skin of berries that developed and ripened without light or under different levels of light intensity. This chapter also describes the relationships between phenolic compounds in the berry skin (anthocyanins, skin tannins and flavonols) of berries that contained the maximum concentration of anthocyanins.

**Berry weight and TSS in berries ripened without sunlight**

Complete bunch shading significantly \((P<0.001)\) reduced berry size of BT, but had no effect on ripening rate and TSS. Reduced size of Cabernet Sauvignon and Pinot Noir berries that developed and ripened without light was observed by Dokoozlian and Kliewer (1996). They hypothesized that a difference in berry size was due to light-mediated effects on cell division and/or cell enlargement. This would include the effect on fruit photosynthesis and carbon metabolism, and activity of photohormones which regulate fruit growth and development (Coombe 1973). A comparison of the berry size between BT (0.96 g) to ST (1.34 g) indicated that bunch shading affected berry development more than canopy shading. At the same sampling date, TSS in berries
developed and ripened without light (BT) was slightly higher than in berries that were highly exposed to sunlight (HET) (Table 5.1). The data suggested that photosynthesis and sugar accumulation was not inhibited in BT berries, which was probably due to the exposure of the majority of leaves to high sunlight intensity. A comparison of TSS in berries of BT and ST indicated that lower juice TSS and delayed ripening for ST may be due to inhibited photosynthesis in shaded leaves (Table 5.1). No difference in TSS of berries from bunches that were fully exposed and shaded to various degrees was also reported by Haselgrove et al. (2000).

5.4.1. The concentration, level and composition of anthocyanins in the berry skin

Concentration, level and composition of anthocyanins
The concentration of total anthocyanins in the skin of Shiraz berries developed and ripened under sunlight intensity greater than 20% of ambient (>300 PPFD) was 1.4-1.5 mg/g berry weight. Increased light intensity above 40% of ambient (800-1500 PPFD) (HET) did not increase accumulation of total and individual anthocyanins when compared to berries that ripen under light intensity of 20-40% of ambient (300-700 PPFD) (MET) (Figure 5.1). Anthocyanins in the mono glucoside form represented 40%; in the acetyl glucoside form 20% while anthocyanins in the coumaroyl glucoside form represented 40% of the total anthocyanin pool (Table 5.2). The major anthocyanin found in the skin of Shiraz grapes was malvidin-3-glucoside and its acetyl and coumaroyl esters (70% of the total anthocyanin pool). Peonidin and its derivatives represented 15%, petunidin 8-10%, delphinidin 5% and cyanidin and its derivatives 1-2%. Similar anthocyanin composition of Shiraz grapes was reported by Boss et al. (1996) and Haselgrove et al. (2000).

A significant reduction (P<0.001) in the concentration of total anthocyanins was observed in the skins of berries that were developed and ripened without light or under low light intensity (<100 PPFD) (50% and 20-30% for BT and ST respectively). The concentration of 0.7 mg/g berry weight anthocyanins in the skin of BT indicated that complete bunch shading had less effect on Shiraz berries than it had on berries of Tokay, Emperor and Cardinal varieties. When bunches of these varieties were developed and ripened in bags no or only a small amount of anthocyanins were
synthesised (Weaver and McCune 1960, Wicks et al. 1982). Dominant anthocyanins of Tokay, Emperor and Cardinal variety were cyanidin and peonidin (Kliewer 1977, Wicks et al. 1982). It was indicated that biosynthesis of cyanidin and peonidin was induced by light thus in the conditions of eliminated sunlight (bags) anthocyanin biosynthesis was inhibited (Wicks et al. 1982). These results suggested that the effect of sunlight may vary between varieties due to their anthocyanin composition and different sensitivity of individual anthocyanins to sunlight.

In the current study, the observed differences between treatments were mainly due to the effect of different levels of light intensity on the accumulation of malvidin and its derivatives as they represented more than 70% of the total anthocyanin pool. It appears that reduced light intensity had a greater effect on malvidin mono-glucoside than on malvidin acetyl-glucoside and malvidin coumaroyl-glucoside (Figure 5.2, 5.4, 5.5) affecting not only the concentrations but also their relative proportions (Table 5.2, 5.3, 5.4). A lower proportion of anthocyanins in the mono-glucoside form and higher proportion of anthocyanins in the coumaroyl-glucoside form in the skins of Shiraz berries ripened under reduced light intensity was also reported by (Haselgrove et al. 2000). It was observed that during development of Shiraz berries, the proportion of malvidin mono-glucoside decreased while the proportion of malvidin coumaroyl-glucoside increased (Boss et al. 1996, Haselgrove et al. 2000). That was due to a lower rate of the accumulation of malvidin mono-glucoside and a greater rate of accumulation of its esters (Boss et al. 1996). The results from this study indicated that reduced intensity of light enhanced malvidin esterification which may be associated with the greater synthesis of $p$-coumaric acid. The concentration of $p$-coumaric acid was significantly higher ($P<0.001$) in the skin of berries developed and ripened without light (BT) or under low light intensity (ST) than in the skins of MET and HET. The concentration of $p$-coumaric acid was as follows: 0.70, 0.53 and 0.43 $\mu$g/g berry weight for ST, MET and HET respectively in the first season, and 0.36, 0.17, 0.18 and 0.40 $\mu$g/g berry weight for ST, MET, HET and BT respectively in the second season. The accumulations of delphinidin and petunidin mono-glucosides were also greatly affected by reduced sunlight intensity. The accumulation of cyanidin and peonidin was affected by sunlight intensity in a similar manner as malvidin and its esters, and reduced light conditions enhanced both the concentration and proportion of cyanidin and peonidin esters (Figure 5.2, 5.4, 5.5).
Relationships between the concentration of anthocyanins and sunlight intensity at the bunch zone

In order to determine if different levels of light intensity had selective effects on individual anthocyanins, their concentrations were plotted against light intensity at the bunch zone (PAR). All relationships were best fitted by a polynomial curve of the second degree. The highest correlation was established for total and individual anthocyanins in the mono-glucoside form, in particular cyanidin and peonidin, indicating that they were the most sensitive to light intensity. High sensitivity of cyanidin and peonidin was also reported by Keller and Hrazdina (1998) who observed four times lower level of cyanidin mono-glucoside and twice lower level of peonidin mono-glucoside in berries of Cabernet Sauvignon grown under sunlight intensity of 2% of ambient compared to berries grown under full sunlight. High correlations were established between the accumulation of total anthocyanins in the acetyl-glucoside form and coumaroyl-glucoside form, and the level of sunlight interception at the bunch zone (Figure 5.5.a and Figure 5.5.b). Furthermore, the concentration of malvidin, delphinidin and petunidin esters showed a closer relationship ($r^2=0.83-94$) to the sunlight intensity than peonidin and cyanidin esters ($r^2=0.55-0.81$).

The data suggested that accumulation of anthocyanins was dependent on the level of sunlight, but it also indicated that the concentration and level of anthocyanins did not increase linearly with the increase of received sunlight at the bunch zone (Figure 5.5.a and Figure 5.5.b) which supported the finding of Mabrouk and Sinoquet (1998). This study indicated that the optimum range of sunlight interception for the maximum accumulation of total anthocyanins or anthocyanins in different forms was around 50% of ambient (800 PPFD). Similar concentrations of anthocyanins were observed in fully exposed berries and in berries of Emperor grapes that were ripened under 46% shading (Kliwer 1977) or 55% shading for Reliance grapes (Gao and Cahoon 1994). In the same experiments when berries of Emperor and Reliance grapes were shaded with 85% or 95% shade cloths respectively, the accumulation of anthocyanins was significantly reduced. Other studies have shown that the maximal accumulation of anthocyanins was observed in the skins of berries grown under relatively low sunlight intensity such as 22% of ambient (<420 PPFD) for Malbec grapes (Kliwer 1977), less than 20% of ambient (<260 PPFD) for Cabernet Sauvignon (Keller and Hrazdina 1998), less than 18% of ambient (<400 PPFD) for greenhouse-grown Pinot Noir (Dookozlian 1990) and
10% of ambient (PPFD not reported) for Merlot (Mabrouk and Sinoquet 1998). However the effects of sunlight interception at the bunch zone comprise not only a light effect, but also a temperature effect. Bunch exposure to sunlight increases the solar heating of the fruit (Smart and Sinclair 1976) and if berry temperature exceeds the optimum range for anthocyanin formation (17-26 °C) (Pirie and Mullins 1977), anthocyanin synthesis would be inhibited (Kliewer and Lider 1970). In the current study, the concentration and composition of anthocyanins was similar in the skin of berries developed and ripened under moderate sunlight intensity (300-700 PPFD) to those developed and ripened under high light intensity (800-1500 PPFD). As the study was conducted in warm climate it was possible that temperature of berries highly exposed to sunlight (HET) exceeded the optimal temperature range and anthocyanin accumulation was inhibited in the HET treatment. A similar effect was observed in berries of Touriga National and Touriga Francesa that were grown at the low altitude under higher temperature conditions compared to berries from vineyards at the higher altitude (Mateus et al. 2002a). However, more information is needed on critical temperature range for anthocyanin biosynthesis and degradation in the skin of Shiraz berries as it was suggested that it may vary with cultivar e.g. anthocyanin biosynthesis was more sensitive to high temperature in Cardinal berries than Pinot Noir (Spayd et al. 2002).

5.4.2. The concentration, level and composition of tannins in the berry skin

The concentration of skin tannins
The accumulation of total skin tannins increased with increasing light intensity (Figure 5.6). In the skin of berries that developed and ripened without light or under low light intensity the concentration (mg/g berry weight) of skin tannins was reduced by 40% (0.38) and 30% (0.41-0.44) respectively of that in the skins of berries grown under 40-80% of ambient (HET) (0.64) (Figure 5.6). Higher sunlight interception increased the accumulation of tannins in the berry skin of HET by 10% (significant at P<0.05) when compared to MET (0.54). This indicated that the accumulation of tannins in the berry skin was less affected by possibly higher temperature of fully exposed berries than the accumulation of anthocyanins. Roubelakis-Angelakis and Kliewer (1986) reported that total phenolic accumulation in the skins of Cardinal berries responded differently to light and temperature than did anthocyanin accumulation. Berries of Pinot Noir grown
in high sunlight conditions accumulated higher concentration of tannins which was also reflected into their responsive wines (Price et al. 1995a). Interestingly the concentration of skin tannins was similar in both seasons which indicated that in this study the accumulation of skin tannins was not affected by season as was the berry size and accumulation of anthocyanins. Kennedy et al. (2002) also reported that the level of skin tannins in Cabernet Sauvignon from a single vineyard was similar in two consecutive years.

The composition of monomers and skin tannins
Epicatechin (48-57%) and epigallocatechin (33-45%) were the most abundant in the extension subunits while catechin represented 47-70% and epicatechin 24-50% of the terminal subunits. The composition of free monomers was predominantly epigallocatechin (33-41%) and epicatechin gallate (25-43%), while catechin (8-18%) and epicatechin (12-21%) remained relatively low. These results are similar to previously reported for Shiraz berries (Kennedy et al. 2001, Downey et al. 2003a). Other studies indicated that the composition of monomers and tannins in the berry skin may be a varietal characteristic (Escribano-Bailon et al. 1995, Moutounet et al. 1996, de Freitas and Glories 1999). Modified light conditions affected not only accumulation but also the composition of tannins in the berry skin as BT and ST had lower proportion of epigallocatechin and higher proportion of epicatechin in extension subunits when compared to MET and HET.

Skin tannins differed to seed tannins by high polymer size (mDP) and the presence of epigallocatechin in the procyanidin structure. The polymer size was significantly lower \( (P<0.001) \) in berries grown without light (19.6 for BT) or under low light intensity (21.9 for ST) compared to 24.0 (for MET and HET) in the skins of berries ripened under high light intensity. The polymer size was similar to previously reported for Shiraz berries by Downey et al. (2003a) and Kennedy et al. (2001). Tannins extracted form both skins and seeds are the principal components of astringency in red wines (Gawel et al. 2001). It was indicated that the composition of tannins and the degree of polymerisation may influence tannin-anthocyanin adducts in wines and thus sensory properties of wines (Cheynier et al. 1997, Cheynier et al. 1998). However it is uncertain whether the small difference of polymer size observed in this study (mDP 20 compared to mDP 24) would
have any impact on phenolic composition of wines and on mouth-feel sensory properties of wines.

*Relationships between the concentration of skin tannins and sunlight intensity at the bunch zone*

The relationship between tannins and received sunlight at the bunch zone was defined as a second degree polynomial relationship. The fitted curves for the accumulation of tannins in the berry skin showed an increasing trend until approximately 80-90% of ambient (>1300 PPFD) (Figure 5.6). This study showed that the concentration of skin tannins is highly dependent on light intensity and that canopy manipulation may affect the accumulation of tannin in the berry skin.

*5.4.3. The concentration and level of quercetin and quercetin-3-glucoside in the berry skin*

The accumulation of quercetin and quercetin-3-glucoside was highly dependent on sunlight interception. No trace of quercetin was observed in the skin of berries developed and ripened without light (BT), while in the skins of berries ripened under low light intensity (ST), the concentration and level of both, quercetin and quercetin-3-glucoside was four times lower than in skins of berries that ripened under higher light intensity (MET and HET) (Figure 5.8). Furthermore, higher light intensity (HET) increased the concentration of quercetin by 30% and quercetin-3-glucoside by 20% compared to MET. These results would indicate that possible high temperature of HET berries (comparing to MET) had little or no effect on the flavonol accumulation. This supports the observation of Spayd *et al.* (2002) that light increased total concentration of flavonols in the skin of Merlot berries, but cooling or heating of bunches had no effect.

The correlation coefficient indicated strong and significant (*P*<0.001) relationships between the concentration and level of quercetin and quercetin-3-glucoside and sunlight interception at the bunch zone (Figure 5.9) which agrees with the studies of Price *et al.* (1995a) and Haselgrove *et al.* (2000). Significant difference (*P*<0.001) was observed in the accumulation of quercetin and quercetin-3-glucoside between seasons and it appears that the seasonal effect was more pronounced for quercetin-3-glucoside (60%) than
quercetin (30% reduction in the second season). The same trend of reduction in the second season was observed in the accumulation of anthocyanins.

5.4.4. Relationships between phenolic compounds in the berry skin and between phenolic compounds in berries and sunlight intensity at the bunch zone

*Relationships between phenolic compounds in the berry skin*

Strong positive correlations established between the concentration of anthocyanins, flavonols and skin tannins (Figure 5.10) indicated that a higher (or lower) concentration of one group of phenolic compounds in the berry skin was correlated with a higher (or lower) concentration of another group of phenolic compounds, while the actual concentrations of these compounds were dependant of sunlight intensity at the bunch zone. The data also supported a general trend of a reduced accumulation of all phenolic compounds in the conditions of low sunlight intensity. However, there was no evidence that the concentration of one group of phenolic compounds can be used to predict the concentration of any other group.

These data were in agreement with previous studies of Merlot (Spayd *et al.* 2002) and Cabernet Franc (Brossaud *et al.* 1999) that showed that berries with a high concentration of total anthocyanins, had also a high concentration of quercetin 3-glucoside. Similarly the concentrations of all phenolic compounds (skin total phenolics, anthocyanins and flavonols) in berries of Cabernet Franc, Merlot and Pinot Noir increased or decreased as a result to applied viticultural treatments of leaf and bunch thinning (Mazza *et al.* 1999). In a study of the effect of water status on the composition of Cabernet Sauvignon, Kennedy *et al.* (2002) reported that treatments with a higher concentration of anthocyanins had also a higher concentration of both flavonols and skin tannins. In other studies, Pinot Noir (Price *et al.* 1995a) and Shiraz bunches (Haselgrove *et al.* 2000) were highly exposed to sunlight intensity, and while an increase of bunch exposure increased the concentration of quercetin-3-glucoside, there was a little or no effect on the concentration of anthocyanins, compared to bunches that were moderately exposed to sunlight. As mention previously it was likely that in the skins of fully exposed berries the temperature effect dominated the light effect as suggested by Iland (1989b).
Relationships between the level of phenolic compounds in skins and seeds, and sunlight intensity at the bunch zone

To determine the effect of sunlight intensity at the bunch zone on the phenolic compounds in a berry, the level (mg/berry) of each group of compounds (anthocyanins, skin tannins and seed tannins) were plotted against PPFD (Figure 5.11). The relationships between the concentration of the different groups of phenolic compounds and sunlight intensity at the bunch zone were defined as second order polynomial relationships. The most affected was the level of anthocyanins and the coefficient of determination ($r^2$) was 0.68 and 0.71 ($P<0.001$) in the first and second season respectively, indicated a strong correlation with PPFD. The fitted curves showed a maximum at 800-900 PPFD, indicating inhibition or degradation of anthocyanins at higher levels of sunlight intensity which may be attributed to the higher temperature of fully exposed berries (Figure 5.11). The level of skin tannins was also highly dependent on PPFD ($r^2$ was 0.63 and 0.58, $P<0.001$, in the first and second season respectively). The fitted curves showed a maximum at 1000-1100 PPFD and then a slight decrease at higher levels of sunlight intensity (>1300 PPFD or >90% of ambient) (Figure 5.11). The least affected was the level of seed tannins (correlation coefficient was 0.18 and 0.48, $P<0.05$, in the first and second season respectively). The fitted curves showed a low correlation with sunlight intensity at the bunch zone (Figure 5.11).

![Figure 5.11. The level of skin tannins, anthocyanins and seed tannins in the berry in relation to sunlight interception at the bunch zone (PPFD). The berries of the following treatments: ST (1-10% sunlight at the bunch zone), MET (10-50% sunlight at the bunch zone) and HET (40-80% sunlight at the bunch zone) were sampled in (a) 2000 and (b) 2001 season. Polynomial curves were fitted for each group of phenolic compounds.](image-url)
Although the limitation of taking measures of sunlight intensity at the bunch zone on a single occasion in each season precluded confident conclusions about the optimal range for maximal accumulation of each group of studied phenolic compounds, the results provided some strong indications about the effect of sunlight intensity on the concentration of phenolic compounds, particularly for berries grown in shaded versus exposed canopies. Nevertheless, this study supported previously reported results that the concentration of phenolic compounds in the berry skin depends on complex interactions between light and temperature effects on the berries. The concentration of anthocyanins and flavonols were affected by season, while no difference was observed in the concentration of skin tannins. Strong relationships established in this study indicated that the accumulation of anthocyanins, tannins and flavonols may be related processes, but their coordination has yet to be established.

5.5. Conclusion

- Sunlight intensity at the bunch zone affected phenolic compounds in the berry skin, including the concentration and level of anthocyanins, skin tannins and flavonols (quercetin and quercetin-3-glucoside).
- Excluded or low sunlight intensity at the bunch zone reduced the accumulation of anthocyanins with an increased proportion of anthocyanins in the coumaroyl-glucoside form relative to anthocyanins in the mono-glucoside form.
- Excluded or low sunlight intensity at the bunch zone reduced the accumulation of tannins in the berry skin and their polymer size.
- Excluded or low sunlight intensity at the bunch zone reduced the accumulation of flavonols (quercetin and quercetin-3-glucoside).
- The accumulation of total anthocyanins did not increase with increasing sunlight intensity above 50% of ambient (~800 PPFD) which could be attributed to possible higher temperature of fully exposed berries. The constituents that were the most sensitive to the light and temperature conditions were anthocyanins in the mono-glucoside form, followed by anthocyanins in the coumaroyl-glucoside form and acetyl-glucoside form.
- The accumulation of skin tannins increased with increasing sunlight intensity at the bunch zone until approximately 80-90% of ambient (>1300 PPFD).
• The accumulation of quercetin and quercetin-3-glucoside increased with increased sunlight intensity at the bunch zone until approximately 60-70% of ambient (900-1200 PPFD), indicating that both compounds were highly dependent on sunlight intensity.

• Strong relationships between the concentration of anthocyanins, tannins and quercetin and quercetin-3-glucoside in the berry skins indicated that accumulation of these compounds paralleled each other, but there is no evidence that a concentration of one phenolic compound (or one group) can be used to predict a concentration of any other compound.
Chapter 6 - Phenolic composition and sensory properties of wines

6.1. Introduction
The phenolic compounds extracted from grape skins and seeds contribute to the sensory properties of wine including wine colour (intensity and stability) and taste (astringency and bitterness) (Jurd and Somers 1970, Haslam 1980, Dallas et al. 1995, Cheynier et al. 1998, de Freitas et al. 2000). They are highly reactive and their reactions commence with the crushing of the grapes and continue through fermentation and maturation. A number of chemical transformations can occur in wines including association of phenolic compounds or their reactions with other compounds such as proteins, polysaccharides and metals, and oxidation-reduction reactions (Haslam 1974, Oszmianski et al. 1985, Dallas et al. 1995, Ricardo de Silva 1997, Cheynier et al. 2000). Polymeric pigments in wines are formed during condensation reactions of anthocyanins with other phenolic compounds and their formation generally depends on the concentration and availability of anthocyanins and tannins to react and produce a variety of pigments from dimers to complex polymers which contribute to the complexity of wine composition and organoleptic properties of the wine. Under the same winemaking conditions (maceration time, temperature, level of alcohol and SO₂), the degree and rate of extraction and diffusion of the phenolic compounds depends on their concentration in skins and seeds, which is associated with variety, vineyard and/or the effects of viticultural practices (Bakker and Timberlake 1985a, Smart et al. 1985b, Ricardo da Silva et al. 1992a, 1992b, Dubois et al. 1996, Farmilo 1997, Goldberg et al. 1998, Hunter 1998, Mazza et al. 1999, de Freitas et al. 2000, Gonzalez-Neves et al. 2001, Esteban et al. 2002, Mateus et al. 2002a).

This chapter describes the relationships between the chemical and phenolic composition of seeds and skins, and the chemical and phenolic composition and sensory properties of wines. Berry and wine composition and their relationships were associated with different levels of sunlight intensity under which berries developed and ripened. The additional treatment, reference treatment (RT), was also included in this experiment on the basis of the previously determined high sensory quality of wines made from RT grapes.
6.2. Material and methods

6.2.1. Sampling procedure, general berry sample preparation and determination of berry colour, skin and seed tannins

Berry and seed samples of ST, MET, HET and RT were collected as previously described in Chapter 2 (page 30). General sample preparation of the berry, skin and seed samples and the methods for determination of berry colour, skin and seed tannins have been described previously in Chapter 4 (pages 60 to 62) and Chapter 5 (pages 92 to 94). It should be noted that while the results in the previous Chapters included eight replicate of each treatment, the results in this chapter include three replicates, obtained by combining all eight replicates into one lot and then subdividing into three winemaking replicates.

6.2.2. Small-lot winemaking procedure and wine analysis

Three small-lot wine replicates per treatment were made according to the standardised winemaking procedure developed at the University of Adelaide. Bunches were de-stemmed and crushed (Zambelli roller de-stemmer 1-2 t/h) with an addition of 40 mg/L SO₂. Must pH was adjusted to 3.6 by adding tartaric acid. After an addition of 100 mg/L DAP the must was inoculated with 0.25 g/L Lalvin strain EC 1118 Saccharomyces Cerevisiae yeast (Lallenmand, Grenaa, Denmark). Wines were fermented for 6 days on skins and were plunged down every 3 and 6 hours during the day and night respectively. Wines were pressed and transferred to glass flagons (5 L for vintage 2000 or 12 L for vintage 2001) equipped with fermentation locks and were kept in a 17°C room for approximately one week until completion of fermentation which was determined by Clinitest and the Rebelein method for reducing sugars (Iland et al. 2000). Wines were racked and cold stabilised at 4°C for 3-4 weeks. Prior to bottling, wines were racked again, pH adjusted to 3.6 and free SO₂ adjusted to 40 ppm (approximately 60 ppm total SO₂). Wines were bottled into 500mL (in 2000) or 750 mL (in 2001) bottles and sealed with screw cap closures. Bottles were stored at 15°C for 12 months until the sensory analysis. Malo-lactic fermentation was not carried out for any of these wines.
6.2.3. Wine chemical analysis

Wine samples for the determination of wine colour and phenolics and HPLC analysis were taken one month after completion of fermentation (at bottling) and after 12 months (at the time of the sensory analysis). Chemical analysis of wines (pH, titratable acidity (TA), free and total SO₂, volatile acidity (VA), malic acid, sugar and alcohol) were performed on wines after 12 months of ageing. The chemical analyses of wines were carried out according to the methods of Iland et al. (2000).

Determination of wine colour and phenolics was carried out according to the method of Somers and Evans (1977) using a Cary Varian UV-visible spectrophotometer and 10 mm, 2 mm and 1 mm quartz cells.

\[ A^{520} \] and \[ A^{420} \]: Absorbance was recorded at 520 nm and 420 nm in a 1 mm quartz cell. The reading was corrected for cuvette path length by multiplying by 10.

\[ A^{\text{acil}} \]: 20 µL of a 10% acetaldehyde solution was added to 2 mL of wine and absorbance at 520 nm and 420 nm was recorded after 45 minutes in a 1 mm quartz cell. The reading was corrected for cuvette path length by multiplying by 10.

\[ A^{\text{SO₂}} \]: 30 µL of a 20% Na₂S₂O₅ was added to 2 mL of wine and absorbance at 520 nm was recorded in a 2 mm quartz cell. The reading was corrected for cuvette path length by multiplying by 5.

\[ A^{\text{HCl}} \]: 100 µL of wine sample was added to 10 mL of 1.0 M HCl. After 3 hours the wine sample was placed in a 10 mm quartz cuvette and absorbance measured at 520 nm and 280 nm. The reading was corrected for dilution by multiplying by 101.

All calculations were performed as outlined in the reference of Somers and Evans (1977).

The concentration of anthocyanins, monomeric flavan-3-ols and tannins, pigmented polymers and tannins was determined by HPLC (Peng et al. 2002). Prior to analysis wines were clarified by centrifugation and filtration (0.45 µm). The HPLC apparatus used was a standard Hewlett-Packard HP 1100 instrument equipped with a diode array detector. The column was a 250 x 4.6 mm polystyrene divinylbenzene reverse phase column (PLRP-S 100 A 5 µm, Polymer Laboratories, Church Stretton, Shropshire, UK) with a guard cartridge (10 x 4.6 mm) packed with the same material. The method
utilised an injection volume of 20 μL, flow rate of 1 mL/min and a binary gradient with mobile phases containing 1.5% w/w H₃PO₄ (solvent A) and 20% v/v solvent A in CH₃CN (solvent B). The elution conditions were: 55 min solvent A decreasing from 92 to 73 %, from 55-59 min solvent A 73%, from 59-64 min solvent A reduced to 30%, from 64-69 min solvent A 30 %, from 70-76% solvent A increased to 92% and then the column was washed and re-equilibrated with 80% solvent B.

The separated individual anthocyanin peaks and pigmented polymer peak were identified by comparison of their relative retention time. Four different concentrations of a commercial standard of malvidin mono-glucoside (M3G) (Extrasynthese, France) were used to construct a calibration curve. One sample of M3G standard was injected after each group of six analysed samples. The absorbance spectra of each anthocyanin peak and a pigmented polymer peak was compared with a standard of malvidin-3-glucoside (M3G) and quantified as M3G equivalents.

Eluting peaks of flavan-3-ols and flavonols were identified by their retention time and spectral comparison to known standards of catechin, epicatechin, epicatechin gallate, quercetin and quercetin-3-glucoside. Catechin, epicatechin and epicatechin gallate standards were purchased from Sigma (Castle Hill, NSW, Australia) and quercetin and quercetin-3-glucoside standards from Extrasynthese (France). Four different concentrations of commercial standards were used to construct a calibration curve. One sample of a standard was injected after each group of 12 analysed samples (catechin standard) and after 24 analysed samples (quercetin-3-glucoside standard). The absorbance spectra of each monomeric flavan-3-ol peaks and a tannin peak was compared with a standard of catechin and quantified as catechin equivalents. Previous studies have found that quercetin-3-glucuronide has similar retention time as quercetin-3-glucoside (Price et al. 1995a, Haselgrove et al. 2000). The employed HPLC method in this study could not resolve quercetin-3-glucuronide from quercetin-3-glucoside, thus the calculated concentrations of quercetin-3-glucoside could be overestimated.
6.2.4. Sensory analysis

descriptive sensory analysis

descriptive sensory analysis of wines undertaken in this study consisted of a series of training and formal sessions. During the training period judges generated appropriate descriptive terms and gained familiarity in recognising and scoring the intensity of selected and defined specific attributes. Some of the attributes were defined by reference standards.

Prior to formal sensory analysis each replicate of each treatment was assessed informally by a panel which consisted of four tasters with extensive experience in wine assessment, in order to assess any artefacts or off-flavours in replicates that might be due to the winemaking process and also to determine the extent of differences among the treatments. In 2000 it was decided to exclude one fermentation replicate due to a strong oxidative flavour. To have the same number of replicates per treatment, the number of replicates of all other treatments was reduced to two. In 2001 all three replicates of each treatment were selected for formal sensory assessment. Some reductive off-flavours were detected in a few wines, and it was decided that all wines be treated with copper sulfate (1.5 mL 1g/L aqueous copper sulfate per 0.7 L bottle) prior to formal assessment.

A tasting panel was convened of 12 male and 2 female staff from the University of Adelaide and the Australian Wine Research Institute (in the first season) and 10 male and 3 female staff (in the second season). All panellists except one in the first and two in the second season had several years' extensive wine sensory descriptive analysis experience. Sensory evaluation comprised the evaluation of mouth-feel attributes in wines and was conducted in the same fashion in both years except for the extension of aroma attributes in addition to mouth-feel attributes in the second season.

The panel attended several training sessions during which panellists discussed and selected by consensus the most appropriate aroma and mouth-feel attributes in the presented wines, following the procedure outlined in Lawless and Heymann (1999). The training period allowed judges to become familiar with the aroma and mouth-feel attributes of the samples as well as to practice scoring the samples. Reference standards for aroma attributes were refined through the sessions (Table 5.1). Finger touch
standards (Table 6.2) as possible representation for astringency sensations experienced in mouth were presented at each session. Training sessions included rating the intensity of the different attributes using a labelled magnitude scale. The last practice session was conducted in isolated booths and judges were presented with one example of each wine in coded glasses, finger touch standards and a finalised set of the reference standards for aroma attributes. The ability of the panellist to consistently identify selected attributes and to consistently rate the intensity of them was monitored through the training sessions.

Table 6.1. Composition of the aroma reference standards.

<table>
<thead>
<tr>
<th>Aroma attributes</th>
<th>Reference standard composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raspberry</td>
<td>One large frozen raspberry in 30 mL base wine*</td>
</tr>
<tr>
<td>Cherry</td>
<td>One fresh cherry (without stone) in 30 mL base wine*</td>
</tr>
<tr>
<td>Plum</td>
<td>Half a canned plum in 30 mL base wine*</td>
</tr>
<tr>
<td>Liquorice</td>
<td>0.5 cm natural liquorice in 30 mL base wine*</td>
</tr>
<tr>
<td>Herbaceous</td>
<td>Two 30mm slices of fresh green beans in 30 mL base wine*</td>
</tr>
<tr>
<td>Straw</td>
<td>Cut straw</td>
</tr>
</tbody>
</table>

*Base wine was a commercial 2 L cask Shiraz wine

Table 6.2. The finger touch standards.

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silky</td>
<td>Silk material</td>
</tr>
<tr>
<td>Velvety</td>
<td>Velvet material</td>
</tr>
<tr>
<td>Coarse/ emery</td>
<td>Emery paper</td>
</tr>
</tbody>
</table>

Formal sessions were conducted daily in isolated booths at 22-23°C under sodium lights (to mask any possible colour difference). All wines were presented in 30 mL samples in 3-digit coded, covered, ISO standard glasses. At each season, all panellists evaluated the same 4 samples (with one example of each treatment) which were in a randomised order across judges. Each fermentation replicate was evaluated in duplicate (2000) and triplicate (2001). Panellists rated aroma attributes and a number of selected mouth-feel attributes (Table 6.3) while holding the wine in mouth and after expectoration.
Table 6.3. The aroma and mouth-feel sensory attributes used for wines made in the (¹) 2001 and (²) 2002 vintage.

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Group</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raspberry</td>
<td>Aroma</td>
<td>Aroma standard</td>
</tr>
<tr>
<td>Cherry</td>
<td>Aroma</td>
<td>Aroma standard</td>
</tr>
<tr>
<td>Plum</td>
<td>Aroma</td>
<td>Aroma standard</td>
</tr>
<tr>
<td>Liquorice</td>
<td>Aroma</td>
<td>Aroma standard</td>
</tr>
<tr>
<td>Herbaceous</td>
<td>Aroma</td>
<td>Aroma standard</td>
</tr>
<tr>
<td>Straw</td>
<td>Aroma</td>
<td>Aroma standard</td>
</tr>
<tr>
<td>Acidity</td>
<td>In mouth</td>
<td>Sourness, tartaric acid solution</td>
</tr>
<tr>
<td></td>
<td>After expectoration</td>
<td></td>
</tr>
<tr>
<td>Silky</td>
<td>In mouth</td>
<td>Fine texture related to the feel of</td>
</tr>
<tr>
<td></td>
<td>After expectoration</td>
<td>silk material</td>
</tr>
<tr>
<td>Velvety</td>
<td>In mouth</td>
<td>Medium-size texture related to the</td>
</tr>
<tr>
<td></td>
<td>After expectoration</td>
<td>feel of velvet material</td>
</tr>
<tr>
<td>Coarse/emery</td>
<td>In mouth</td>
<td>Rough texture related to the feel of</td>
</tr>
<tr>
<td></td>
<td>After expectoration</td>
<td>fine emery paper</td>
</tr>
<tr>
<td>Grainy</td>
<td>In mouth</td>
<td>Feeling of the presence of particulate</td>
</tr>
<tr>
<td></td>
<td>After expectoration</td>
<td>matter, grainy, on mouth surface</td>
</tr>
<tr>
<td>Drying</td>
<td>In mouth</td>
<td>Feeling of lack of lubrication or</td>
</tr>
<tr>
<td></td>
<td>After expectoration</td>
<td>dehydration in mouth</td>
</tr>
<tr>
<td>Hotness</td>
<td>In mouth</td>
<td>A high degree of warmth, irritation</td>
</tr>
<tr>
<td>Fullness</td>
<td>In mouth</td>
<td>A feeling of full mellow sensation in</td>
</tr>
<tr>
<td></td>
<td></td>
<td>the mouth</td>
</tr>
<tr>
<td>Puckery</td>
<td>After expectoration</td>
<td>Involuntary reflex action of mouth</td>
</tr>
<tr>
<td></td>
<td></td>
<td>surface being brought together and</td>
</tr>
<tr>
<td></td>
<td></td>
<td>released in an attempt to lubricate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mouth surfaces</td>
</tr>
<tr>
<td>Adhesive</td>
<td>After expectoration</td>
<td>A feeling that mouth surfaces are</td>
</tr>
<tr>
<td></td>
<td></td>
<td>adhering or sticking to one another</td>
</tr>
<tr>
<td>Overall fruit</td>
<td>After expectoration</td>
<td>Overall amount of flavour impression</td>
</tr>
<tr>
<td>flavour</td>
<td></td>
<td></td>
</tr>
<tr>
<td>persistence</td>
<td>After expectoration</td>
<td>Length of perception of fruit flavour</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Astringent persistence</td>
<td>After expectoration</td>
<td>Length of perception of any astringent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sensation</td>
</tr>
</tbody>
</table>

The reference standards for wine aroma (in covered wine tasting glasses) (Table 6.1) and finger touch standards (Table 6.2) were presented at each session. Panellists could refer to standards before rating the samples and at any time during the session. Panellists rated the intensity of aroma and mouth-feel attributes on a labelled magnitude
line scale (Green 1993) from detectable to weak, moderate, strong, very strong and the strongest imaginable. Panellists rinsed thoroughly with distilled water and had a rest of 30 seconds between samples. Data acquisition was carried out using the software Fizz (Version 1.3, Biosystemes, Couternon, France).

**Quality rating**
Rating wines on a 20-point quality scale (Rankine 1986) required “expert” judges, thus 15 judges were selected among the University of Adelaide and the Australian Wine Research Institute staff, based on their previous sensory experience and knowledge. Rating wines on a 20-point quality scale was conducted in isolated booths under natural light. Panellists were asked to rate wine appearance on a scale of 1-3 points, wine aroma 1-7 points and palate (flavour by mouth) 1-10 points. Sensory evaluation of one replicate of each treatment was repeated twice in the first and three times in the second season.

**Preference test**
Preference test was used to indicate which sample was preferred. The “expert” panellists were asked to rank wines on a preference basis from 1 to 4, where 1 was the most preferable and 4 the least. Sensory evaluation of one replicate of each treatment was repeated twice in the first and three times in the second season.

**Red wine colour and purple hue assessment**
Intensity of red colour and purple hue of wines from 2001 season was evaluated under natural light against a white background. At two sessions, each panellist was presented with all replicates of all treatments in a randomised order, so that all replicates were rated in duplicate. Panellists rated the intensity of red colour and purple hue on a 10-point unstructured line scale (for each colour), from none to high.

**6.2.5. Statistical methods for sensory analysis**
Each attribute rated by judges was subjected to analysis of variance (ANOVA) to ascertain the effects of sample, judge and replicate, using a mixed model ANOVA and treating judges in a random effect. The method used to discriminate among the means was Fisher’s least significant difference procedures, P<0.05. Principal component
analysis was performed using the correlation matrix of the mean values for each sample, with no rotation. The data from the preference test were analysed by the multiple comparison ranking procedure (Newell and MacFarlane 1987). Statistical analyses were performed with Fizz (Version 1.3, Biosystemes, Couternon, France), Genstat (Version 5, Lawes Agricultural Trust, Rothamsted Experimental Station, UK) and JMP (Version 3.1, SAS Institute, Cary, NC, USA).

6.3. Results

In the first season the fruit from the ST and RT treatment was picked 7 days later than the fruit from the MET and HET treatment, aiming for a stage of berry ripeness at harvest of 23-24 °Brix. In the second season MET, HET and RT were picked the same day and ST 7 days later, aiming for a stage of berry ripeness at harvest of 24-25 °Brix.

As previously mentioned, the samples used for winemaking were obtained by combining the fruit of eight replicates of each treatment into one lot and then subdividing into three replicates for each treatment. Chapter 5 describes phenolic compounds in the berry skin of ST, MET and HET treatments in two seasons (1999/2000) and 2000/2001). All berry samples, except MET and HET in the first season correspond to the winemaking samples. The samples of MET and HET, that contained the maximum concentration of anthocyanins, were collected one week later than the winemaking samples. Thus the summary of chemical and phenolic composition of seeds and skins of berries collected for winemaking, including the additional treatment RT, is given in the Table 6.

6.3.1. Chemical composition of berries sampled for winemaking

6.3.1.1. Chemical composition of berries

The difference in berry size between ST, MET and HET was described previously in Chapter 3. Berry size of the RT treatment was significantly ($P<0.001$) lower (30%) than the other treatments (Table 6.4). In the second season the berry size was reduced by 20% and seed weight by 25% for all treatments compared to the first season (Table 6.4). The values of berry juice pH, titratable acidity (TA), concentration of total anthocyanins
and skin total phenolics (as determined by UV/visible spectroscopy, the method described on page 62) are given in Table 6.4.

Table 6.4. Berry weight, seed weight, seed number, juice total soluble solids (°Brix), juice pH and the concentration of total anthocyanins and skin total phenolics of berries at harvest in the 2000 and 2001 season. Each value represents the mean of 3 replicates. Means in columns followed by different letters are significantly different within one season.

<table>
<thead>
<tr>
<th>Treat.</th>
<th>Year</th>
<th>Berry weight (g)</th>
<th>Seed weight (mg)</th>
<th>No of seeds/ berry</th>
<th>°Brix</th>
<th>pH</th>
<th>TA</th>
<th>Anthocyanins (mg/g berry weight)</th>
<th>Skin phenolics (au/g berry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST</td>
<td>2000</td>
<td>1.30 b</td>
<td>32.8 b</td>
<td>1.7</td>
<td>24.0 b</td>
<td>3.55 b</td>
<td>9.7 b</td>
<td>1.19 a</td>
<td>0.75 a</td>
</tr>
<tr>
<td>MET</td>
<td>2000</td>
<td>1.32 b</td>
<td>30.0 a</td>
<td>1.8</td>
<td>23.2 a</td>
<td>3.46 a</td>
<td>9.3 b</td>
<td>1.23 a</td>
<td>0.74 a</td>
</tr>
<tr>
<td>HET</td>
<td>2000</td>
<td>1.40 c</td>
<td>30.5 a</td>
<td>1.8</td>
<td>23.1 a</td>
<td>3.45 a</td>
<td>8.9 ab</td>
<td>1.14 a</td>
<td>0.74 a</td>
</tr>
<tr>
<td>RT</td>
<td>2000</td>
<td>1.08 a</td>
<td>29.5 a</td>
<td>1.7</td>
<td>23.4 a</td>
<td>3.60 b</td>
<td>8.4 a</td>
<td>1.64 b</td>
<td>0.99 b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>***</td>
<td>*</td>
<td>ns</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>ST</td>
<td>2001</td>
<td>1.20 c</td>
<td>23.7</td>
<td>2.7</td>
<td>25.2 b</td>
<td>3.82 b</td>
<td>7.7</td>
<td>1.20 a</td>
<td>0.72 a</td>
</tr>
<tr>
<td>MET</td>
<td>2001</td>
<td>1.13 b</td>
<td>23.5</td>
<td>2.8</td>
<td>24.3 a</td>
<td>3.68 a</td>
<td>7.5</td>
<td>1.46 b</td>
<td>0.83 b</td>
</tr>
<tr>
<td>HET</td>
<td>2001</td>
<td>1.18 b</td>
<td>23.4</td>
<td>2.6</td>
<td>24.7 a</td>
<td>3.69 a</td>
<td>7.6</td>
<td>1.44 b</td>
<td>0.84 b</td>
</tr>
<tr>
<td>RT</td>
<td>2001</td>
<td>0.89 a</td>
<td>22.7</td>
<td>2.9</td>
<td>24.5 a</td>
<td>3.78 b</td>
<td>7.4</td>
<td>1.99 c</td>
<td>1.19 c</td>
</tr>
<tr>
<td></td>
<td></td>
<td>***</td>
<td>ns</td>
<td>ns</td>
<td>**</td>
<td>***</td>
<td>ns</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

au: absorbance units
ns not significant, *,**,*** indicate significance at P<0.05, P<0.01, P<0.001 respectively

6.3.1.2. The concentration of anthocyanins in the berry skin
The concentration (mg/g berry weight) of total anthocyanins, as determined by HPLC, was as follows 1.04, 1.11, 1.11, 1.64 and 0.93, 1.21, 1.34 and 1.74 mg/g berry weight for ST, MET, HET and RT in the first season and second season respectively (Figure 6.1). The composition of anthocyanins in the berry skin differed between treatments and the proportion of anthocyanins in the mono-glucoside form, acetyl-glucoside and coumaroyl-glucoside form for the treatments was as follows: 38%, 20%, 42% and 31%, 23%, 46% in the skins of ST; 43%, 20%, 37% and 37%, 21%, 43% in the skins of MET; 43%, 20%, 36% and 37%, 21%, 42% in the skin of HET and 48%, 20%, 32% and
43%, 21%, 36% in the skin of RT in the first and second season respectively. Reduced light conditions for ST decreased the concentration of anthocyanins in the monoglucoside form but increased the proportion of anthocyanins in the coumaroyl-glucoside form, while the proportion of anthocyanins in the acetyl-glucoside form was similar to those of MET and HET. When comparing between seasons, in the first season all treatments had slightly higher proportion of anthocyanins in the mono-glucoside form, but lower proportion of anthocyanins in the coumaroyl-glucoside form, while the proportion of anthocyanins in the acetyl glucoside form was similar in both years.

Figure 6.1. The concentration of (a) total anthocyanins, (b) anthocyanins in the mono-glucoside form, (c) anthocyanins in the acetyl-glucoside form and (d) anthocyanins in the coumaroyl-glucoside form in the berry skin at harvest. The berries of the following treatments: ST (1-10% sunlight at the bunch zone), MET (10-50% sunlight at the bunch zone), HET (40-80% sunlight at the bunch zone) and RT were sampled for winemaking in the 2000 and 2001 season. Error bars represent ± standard error of the mean (n=3). Different letters above error bars indicate significant difference (P<0.05) between means within one season. Note the different y-axis scales.
6.3.1.3. The concentration of monomers and tannins in the berry skin

Low bunch exposure for ST reduced the concentration (mg/g berry weight) of total monomer flavan-3-ols and skin tannins in the berry skin when compared to MET, HET and RT. The concentration of tannins in the berry skin of ST (0.37 and 0.42) was lower by 30% when compared to MET (0.55 and 0.68) and HET (0.50 and 0.63) and 50% when compared to RT (0.70 and 0.93 in the first and the second season respectively) (Figure 6.2 a). The same differences were shown in the concentration of extension subunits in the berry skin with ST significantly lower ($P<0.001$) and RT significantly higher ($P<0.001$) than the other two treatments.

Figure 6.2. The concentration of (a) total tannins, (b) extension subunits, (c) terminal subunits and (d) monomeric flavan-3-ols in the berry skin at harvest. The berries of the following treatments: ST (1-10% sunlight at the bunch zone), MET (10-50% sunlight at the bunch zone), HET (40-80% sunlight at the bunch zone) and RT were sampled for winemaking in the 2000 and 2001 season. Error bars represent ± standard error of the mean (n=3). Different letters above error bars indicate significant difference ($P<0.05$) between means within one season. Note the different y-axis scales.
Although MET had slightly higher concentration of total skin tannins and extension units than HET, there was no significant difference \((P<0.05)\) between MET and HET (Figure 6.2 b). The concentration of terminal subunits showed variation between treatments and seasons. In the first season all treatments has similar concentration of terminal subunits in the berry skin (0.036-0.046) while in the second season ST (0.050 mg) and MET (0.052) was significantly lower \((P<0.001)\) than HET (0.064) and RT (0.071) (Figure 6.2.c). The concentration of monomer flavan-3-ols was significantly \((P<0.001)\) lower in the skins of the ST treatment (0.013 and 0.009) than MET and HET (0.016 and 0.014) and significantly higher \((P<0.001)\) in the skins of the RT treatment (0.022 and 0.024 in the first and second season respectively), than the other treatments (Figure 6.2.d). The proportion and composition of extension, terminal and monomer subunits in berry skins at harvest was similar to that previously described in Chapter 5 (Table 5.6).

6.3.1.4. The concentration of flavonols in the berry skin

The concentration (mg/g berry weight) of flavonols (quercetin and quercetin-3-glucoside) was significantly different \((P<0.001)\) in the skin of different treatments (Figure 6.3).

Figure 6.3. The concentration of (a) quercetin-3-glucoside and (b) quercetin in the berry skin at harvest. The berries of the following treatments: ST (1-10% sunlight at the bunch zone), MET (10-50% sunlight at the bunch zone), HET (40-80% sunlight at the bunch zone) and RT were sampled for winemaking in the 2000 and 2001 season. Error bars represent \(±\) standard error of the mean \((n=3)\). Different letters above error bars indicate significant difference \((P<0.05)\) between means within one season. Note the different y-axis scales.
The concentration (mg/g berry weight) of quercetin-3-glucoside was as follows: 0.020 and 0.020 (ST), 0.013 and 0.009 (MET), 0.016 and 0.011 (HET) and 0.032 and 0.029 mg/gram berry weight (RT) in the first and second season respectively (Figure 6.3). The concentration (mg/g berry weight) of quercetin was as follows: 0.0003 and 0.0003 (ST), 0.0016 and 0.0012 (MET), 0.0026 and 0.0020 (HET) and 0.0048 and 0.0048 mg/g berry weight (RT) in the first and second season respectively (Figure 6.3).

6.3.1.5. The concentration of monomers and tannins in seeds
The concentration (mg/g berry weight) of seed tannins was not affected by treatments or seasons despite the differences in the berry size, seed size and seed number. All treatments had similar concentration of seed tannins of 1.6-1.9 mg/g berry weight (Figure 6.4 a). Similar trends were observed in the concentration of extension and terminal subunits, except for the lower concentration of terminal subunits for ST in the first season (Figure 6.4.c). The concentration of free monomers was significantly ($P<0.001$) lower in the seeds of RT in both seasons, while there was no difference in the concentration of free monomers in the seeds of the ST, MET and HET treatments. The composition of seed tannins, extension and terminal subunits and flavan-3-ol monomers was similar to that previously described in Chapter 3.
Figure 6.4. The concentration of (a) total tannins, (b) extension subunits, (c) terminal subunits and (d) monomeric flavan-3-ol in seeds of berries at harvest. The berries of the following treatments: ST (1-10% sunlight at the bunch zone), MET (10-50% sunlight at the bunch zone), HET (40-80% sunlight at the bunch zone) and RT were sampled for winemaking in the 2000 and 2001 season. Error bars represent ± standard error of the mean (n=3). Different letters above error bars indicate significant difference (P<0.05) between means within one season. Note the different y-axis scales.

6.3.1.6. Ratio between phenolic compounds in grape seeds and skins

In summary, the concentration of total anthocyanins was similar or lower in berries of the ST treatment, comparing to berries of MET and HET treatments. Shading enhanced the proportion of anthocyanins in the coumaroyl-glucoside form, relative to anthocyanins in the mono-glucoside form. RT was significantly higher in the concentration of total anthocyanins and anthocyanins in different forms compared to the other treatments. The concentration of skin monomeric flavan-3-ols and tannins was significantly (P<0.001) lower in ST, while significantly higher in RT (P<0.001) than in the other treatments. The concentration of seed tannins was similar in all treatments.
The ratios between the concentration of skin or seed tannins and the concentration of anthocyanins are given in Table 6.5. The ST treatment had the lowest ratio of skin tannins to anthocyanins and the highest ratio of seed tannins to anthocyanins; the opposite trend was apparent for the RT treatment. The MET treatment had both ratios higher than the HET treatment (Table 6.5).

Table 6.5. Ratio between the concentrations of skin and seed tannins, and anthocyanins in berries at harvest. The berries of the ST, MET, HET and RT treatment were sampled for winemaking in the 2000 and 2001 season. Each value represents the mean of 3 replicates. Means in columns followed by different letters are significantly different within one season.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Season</th>
<th>Skin tannins/anthocyanins</th>
<th>Seed tannins/anthocyanins</th>
<th>(Skin tannins + seed tannins)/anthocyanins</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST</td>
<td>2000</td>
<td>0.37 a</td>
<td>1.79 b</td>
<td>2.16 b</td>
</tr>
<tr>
<td>MET</td>
<td>2000</td>
<td>0.49 c</td>
<td>1.70 b</td>
<td>2.19 b</td>
</tr>
<tr>
<td>HET</td>
<td>2000</td>
<td>0.43 b</td>
<td>1.61 b</td>
<td>2.04 b</td>
</tr>
<tr>
<td>RT</td>
<td>2000</td>
<td>0.48 c</td>
<td>1.16 a</td>
<td>1.64 a</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>**</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>ST</td>
<td>2001</td>
<td>0.45 a</td>
<td>1.94 d</td>
<td>2.40 c</td>
</tr>
<tr>
<td>MET</td>
<td>2001</td>
<td>0.56 b</td>
<td>1.40 c</td>
<td>1.96 b</td>
</tr>
<tr>
<td>HET</td>
<td>2001</td>
<td>0.47 a</td>
<td>1.22 b</td>
<td>1.69 a</td>
</tr>
<tr>
<td>RT</td>
<td>2001</td>
<td>0.60 b</td>
<td>1.01 a</td>
<td>1.61 a</td>
</tr>
<tr>
<td></td>
<td>2001</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
</tbody>
</table>

ns not significant, *, **, *** indicate significance at P<0.05, P<0.01, P<0.001 respectively

6.3.2. Chemical composition of wines

6.3.2.1. Chemical composition of wines after fermentation
The concentration of anthocyanins

In the text, the term “after fermentation” is used for the wines analysed at bottling time, which was approximately one month after completion of fermentation. The term “after 12 months of ageing” is used for wines that aged for approximately 12 months after bottling, and then they were analysed for chemical composition and sensory properties.
In the 2000 season there was no significant difference \((P<0.001)\) in the concentration of total anthocyanins in wines after fermentation (as determined by the HPLC method) between ST, MET and HET, while in the 2001 season ST treatment was lower when compared to MET and HET (Figure 6.5). The same differences were observed in the concentration of anthocyanins in the mono-glucoside form, anthocyanins in the acetyl-glucoside form and anthocyanins in the coumaroyl-glucoside form (Figure 6.5). In both seasons the RT treatment had significantly \((P<0.001)\) higher concentration of total anthocyanins, anthocyanins in the mono-glucoside form and anthocyanins in the acetyl-glucoside form, while the concentration of anthocyanins in the coumaroyl-glucoside form was not significantly different \((P<0.001)\) (Figure 6.5).
Figure 6.5. The concentration of (a) total anthocyanins, (b) anthocyanins in the mono-glucoside form, (c) anthocyanins in the acetyl-glucoside form and (d) anthocyanins in the coumaroyl-glucoside form in wines after fermentation and after 12 months of ageing. Wines were made from berries of the following treatments: ST (1-10% sunlight at the bunch zone), MET (10-50% sunlight at the bunch zone), HET (40-80% sunlight at the bunch zone) and RT in the 2000 and 2001 season. Error bars represent ± standard error of the mean (n=3). Different letters above error bars indicate significant difference (P<0.05) between means within one season. Note the different y-axis scales.
In both seasons ST, MET and HET had a similar proportion of anthocyanins in the mono-glucoside form, acetyl-glucoside form, and coumaroyl-glucoside from but when compared to RT, they had a significantly \( P<0.001 \) lower proportion of anthocyanins in the mono-glucoside form and higher proportion of anthocyanins in the coumaroyl-glucoside form (Table 6.6).

Table 6.6. Proportion of anthocyanins in the mono-glucoside form, acetyl-glucoside form and coumaroyl-glucoside form in wines from the 2000 and 2001 season after fermentation and after 12 months of ageing. Each value represents the mean of 3 fermentation replicates. Means in columns followed by different letters are significantly different within one season.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Year</th>
<th>After fermentation</th>
<th>After 12 months of ageing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total mono-glucoside (%)</td>
<td>Total acetyl-glucoside (%)</td>
</tr>
<tr>
<td>ST</td>
<td>2000</td>
<td>57.2 a</td>
<td>32.4 b</td>
</tr>
<tr>
<td>MET</td>
<td>2000</td>
<td>58.4 a</td>
<td>30.8 b</td>
</tr>
<tr>
<td>HET</td>
<td>2000</td>
<td>59.1 a</td>
<td>30.5 b</td>
</tr>
<tr>
<td>RT</td>
<td>2000</td>
<td>63.5 b</td>
<td>27.7 a</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>ST</td>
<td>2001</td>
<td>58.1 a</td>
<td>33.2 b</td>
</tr>
<tr>
<td>MET</td>
<td>2001</td>
<td>60.0 ab</td>
<td>30.5 a</td>
</tr>
<tr>
<td>HET</td>
<td>2001</td>
<td>60.0 ab</td>
<td>30.8 a</td>
</tr>
<tr>
<td>RT</td>
<td>2001</td>
<td>63.2 b</td>
<td>30.0 a</td>
</tr>
<tr>
<td></td>
<td>2001</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

* *** indicate significance at \( P<0.05 \), \( P<0.01 \), \( P<0.001 \) respectively

The concentration of flavan-3-ols and flavonols
The concentration of total monomeric flavan-3-ols was significantly \( P<0.001 \) higher in ST, MET and HET wines after fermentation (33-35 mg/L and 53-62 mg/L) than in RT wines (26 mg/L and 40 mg/L in the first and second season respectively) (Figure 6.6). The concentration of total flavan-3-ols was almost twice as high in the second than in the first season (Figure 6.6).
In wines after fermentation, the concentration (mg/L) of quercetin-3-glucoside was as follows: 11.5, 17.0, 17.6 and 20.0 for ST, MET, HET and RT in the first season and 7.8, 8.8, 8.6 and 8.5 mg/L for ST, MET, HET and RT in the second season (Figure 6.7). It should be noted that under the HPLC conditions used in this study quercetin glucuronide may be eluted together with quercetin-3-glucoside.

The concentration (mg/L) of quercetin was as follows: 0.2, 1.4, 2.5 and 2.3 mg/L for ST, MET, HET and RT in the first season and 0.6, 1.7, 2.0 and 2.2 mg/L for ST, MET, HET and RT in the second season (Figure 6.7). The concentrations of both quercetin-3-glucoside and quercetin was significantly ($P<0.001$) lower in wines of ST and higher in wines of RT than in wines of the other treatments (Figure 6.7).
The concentration of polymeric pigments and tannins

In the 2000 season the concentration of polymeric pigments in wines (expressed as mg M3G equivalents/L), as determined by the HPLC method, was similar for ST, MET and HET treatments (52-62 mg/L) whilst RT was significantly (P<0.001) higher (103 mg/L) (Figure 6.5). In the 2001 season ST had significantly lower (P<0.001) concentration of polymeric pigments (68 mg/L), RT was significantly higher (P<0.001) (175 mg/L) than MET (118 mg/L) and HET (104 mg/L). When compared between seasons, in the second season the concentration of polymeric pigments was 15% higher for ST, 100% for MET and HET and 75% for RT (Figure 6.8).
Figure 6.8. The concentration of polymeric pigments in wines after fermentation and after 12 months of ageing. Wines were made from berries of the following treatments: ST (1-10% sunlight at the bunch zone), MET (10-50% sunlight at the bunch zone), HET (40-80% sunlight at the bunch zone) and RT in the 2000 and 2001 season. Error bars represent ± standard error of the mean (n=3). Different letters above error bars indicate significant difference (P<0.05) between means within one season.

In the season 2000 the concentration (mg catechin equivalents/L) of tannins, as determined by the HPLC method at 280 nm, was similar for ST, MET and HET (278-340 mg/L), but significantly higher (P<0.001) for RT (535 mg/L) (Figure 6.9). In the following 2001 season ST was significantly lower (304 mg/L), compared to MET (592 mg/L) and HET (607 mg/L) and RT (803 mg/L) which was significantly higher (P<0.001) than the other treatments (Figure 6.9). When comparing between seasons, in the second season, the concentration of tannins was higher by 10% for ST, 45% for MET and HET and 30% for RT than in the first season.

Figure 6.9. The concentration of tannins in wines after fermentation and after 12 months of ageing. Wines were made from berries of the following treatments: ST (1-10% sunlight at the bunch zone), MET (10-50% sunlight at the bunch zone), HET (40-80% sunlight at the bunch zone) and RT in the 2000 and 2001 season. Error bars represent ± standard error of the mean (n=3). Different letters above error bars indicate significant difference (P<0.05) between means within one season.
Modified wine colour density and wine hue

Wine colour density (1) was calculated as the sum of the absorbance at 520 nm and 420 nm at wine pH. The parameter wine colour density at pH 3.60 (2) was calculated as the sum of the absorbance at 520 nm and 420 nm at adjusted wine pH to 3.60. Modified wine colour density (3) was calculated as the sum of the absorbance at 520 nm and 420 nm, after the addition of acetaldehyde to the wine samples to allow for the effect of sulphur dioxide concentrations, at adjusted wine pH to 3.60 (Table 6.7).

Table 6.7. Wine colour density at wine pH (1), wine colour density at adjusted wine pH to 3.60 (2) and wine colour density at adjusted wine pH to 3.60 and the addition of acetaldehyde (3) of wines after fermentation and after 12 months of ageing. Each value represents the mean of 3 fermentation replicate. Means in rows followed by different letters are significantly different.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Year</th>
<th>Wine pH</th>
<th>Wine colour density (wine pH) 1</th>
<th>Wine colour density (pH=3.60) 2</th>
<th>Modified wine colour density (pH=3.60 and acetaldehyde) 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>After fermentation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST</td>
<td>2000</td>
<td>3.56</td>
<td>7.58 b</td>
<td>5.27 a</td>
<td>7.11 b ***</td>
</tr>
<tr>
<td>MET</td>
<td>2000</td>
<td>3.53</td>
<td>10.11 b</td>
<td>8.86 a</td>
<td>9.62 ab **</td>
</tr>
<tr>
<td>HET</td>
<td>2000</td>
<td>3.50</td>
<td>10.19 c</td>
<td>7.49 a</td>
<td>9.62 b ***</td>
</tr>
<tr>
<td>RT</td>
<td>2000</td>
<td>3.57</td>
<td>19.56 b</td>
<td>13.95 a</td>
<td>19.02 b ***</td>
</tr>
<tr>
<td>ST</td>
<td>2001</td>
<td>3.50</td>
<td>8.51 c</td>
<td>5.22 a</td>
<td>7.98 b ***</td>
</tr>
<tr>
<td>MET</td>
<td>2001</td>
<td>3.34</td>
<td>13.99 c</td>
<td>8.70 a</td>
<td>12.13 b ***</td>
</tr>
<tr>
<td>HET</td>
<td>2001</td>
<td>3.55</td>
<td>12.07 b</td>
<td>8.01 a</td>
<td>11.73 b ***</td>
</tr>
<tr>
<td>RT</td>
<td>2001</td>
<td>3.43</td>
<td>22.40 c</td>
<td>10.45 a</td>
<td>14.37 b ***</td>
</tr>
<tr>
<td>After 12 months of ageing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST</td>
<td>2000</td>
<td>3.29</td>
<td>8.42 b</td>
<td>6.84 a</td>
<td>7.23 a **</td>
</tr>
<tr>
<td>MET</td>
<td>2000</td>
<td>3.38</td>
<td>12.33 b</td>
<td>10.17 a</td>
<td>10.32 a ***</td>
</tr>
<tr>
<td>HET</td>
<td>2000</td>
<td>3.32</td>
<td>10.39 b</td>
<td>8.72 a</td>
<td>8.98 a **</td>
</tr>
<tr>
<td>RT</td>
<td>2000</td>
<td>3.40</td>
<td>16.27 b</td>
<td>14.37 a</td>
<td>14.58 a ***</td>
</tr>
<tr>
<td>ST</td>
<td>2001</td>
<td>3.43</td>
<td>6.87 b</td>
<td>5.40 a</td>
<td>6.40 b **</td>
</tr>
<tr>
<td>MET</td>
<td>2001</td>
<td>3.30</td>
<td>11.61 c</td>
<td>8.96 a</td>
<td>9.67 b ***</td>
</tr>
<tr>
<td>HET</td>
<td>2001</td>
<td>3.43</td>
<td>11.21 b</td>
<td>9.08 a</td>
<td>10.18 a **</td>
</tr>
<tr>
<td>RT</td>
<td>2001</td>
<td>3.36</td>
<td>18.65 c</td>
<td>15.14 a</td>
<td>16.19 b ***</td>
</tr>
</tbody>
</table>

*, **, *** indicate significance at P<0.05, P<0.01, P<0.001 respectively
The results in Table 6.7 showed that the values of wine colour density were significantly different in response to wine pH and sulphur dioxide concentrations. Modified wine colour density (3) was considered as the most appropriate measure, due to the elimination of any effect of sulphur dioxide and pH on the colour density of the wines, as suggested by Iland (2001). In this study the wines after fermentation and after 12 months of ageing were compared on a basis of modified wine colour density.

To determine if these differences were similar in young red wines as they were in wines after 12 months of ageing, the values of wine colour density at pH 3.60 (2) and modified wine colour density (3) of wine after fermentation and after 12 months of ageing were plotted against wine colour density determined at wine pH (Figure 6.10).

![Figure 6.10. The relationship between wine colour density at wine pH, and wine colour density at adjusted wine pH to 3.60 and modified wine colour density at adjusted wine pH to 3.60 and an addition of acetaldehyde, measured and calculated for wines after fermentation and after 12 months of ageing.](image)

The linear regression equations and coefficients of determination indicated highly significant relationships between wine colour density (at actual wine pH) (1) and, wine colour density at pH 3.60 (2) and modified wine colour density (3) (an addition of acetaldehyde and pH adjusted to 3.60) (Figure 6.10). Higher values for modified colour density in wines after the addition of acetaldehyde were due to the contribution of anthocyanin component previously bleached by SO₂. The effect of sulphur dioxide was greater in wines after fermentation due to a higher proportion of monomeric
anthocyanins. During wine ageing the concentration of monomeric anthocyanins declined and the effect of sulphur dioxide became less pronounced, thus after 12 months of ageing the difference between modified wine colour density (an addition of acetaldehyde and pH adjusted to 3.60) and wine colour density (pH adjusted to 3.60) diminished.

Table 6.8. Modified wine colour density, modified wine hue, concentration of pigmented polymers, total anthocyanins and total phenolics in wines after fermentation and after 12 months of ageing. Each value represents the mean of 3 fermentation replicates. Means in columns followed by different letters are significantly different within one season.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Year</th>
<th>Modified wine density</th>
<th>Modified wine hue</th>
<th>Total anthocyanins (mg/L)</th>
<th>Total phenolics (au/L)</th>
<th>Polymeric pigments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>After fermentation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST</td>
<td>2000</td>
<td>7.1 a</td>
<td>0.49 c</td>
<td>430.2 a</td>
<td>29.1 a</td>
<td>1.1 a</td>
</tr>
<tr>
<td>MET</td>
<td>2000</td>
<td>9.6 b</td>
<td>0.47 b</td>
<td>462.6 a</td>
<td>31.4 ab</td>
<td>1.7 c</td>
</tr>
<tr>
<td>HET</td>
<td>2000</td>
<td>9.6 b</td>
<td>0.46 b</td>
<td>486.4 a</td>
<td>33.0 b</td>
<td>1.4 b</td>
</tr>
<tr>
<td>RT</td>
<td>2000</td>
<td>19.0 c</td>
<td>0.37 a</td>
<td>787.3 b</td>
<td>51.4 c</td>
<td>2.4 d</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>***</td>
<td>**</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>ST</td>
<td>2001</td>
<td>8.0 a</td>
<td>0.51 c</td>
<td>405.3 a</td>
<td>28.7 a</td>
<td>1.0 a</td>
</tr>
<tr>
<td>MET</td>
<td>2001</td>
<td>12.1 b</td>
<td>0.45 a</td>
<td>569.3 b</td>
<td>39.8 b</td>
<td>1.5 b</td>
</tr>
<tr>
<td>HET</td>
<td>2001</td>
<td>11.7 b</td>
<td>0.47 b</td>
<td>554.1 b</td>
<td>39.9 b</td>
<td>1.4 b</td>
</tr>
<tr>
<td>RT</td>
<td>2001</td>
<td>14.4 c</td>
<td>0.47 b</td>
<td>642.9 c</td>
<td>45.1 c</td>
<td>1.8 c</td>
</tr>
<tr>
<td></td>
<td>2001</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td><strong>After 12 months of ageing</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST</td>
<td>2000</td>
<td>7.23 a</td>
<td>0.61 b</td>
<td>173.1 b</td>
<td>23.4 a</td>
<td>2.0 a</td>
</tr>
<tr>
<td>MET</td>
<td>2000</td>
<td>10.32 c</td>
<td>0.59 a</td>
<td>123.4 a</td>
<td>25.2 b</td>
<td>3.7 b</td>
</tr>
<tr>
<td>HET</td>
<td>2000</td>
<td>8.98 b</td>
<td>0.61 b</td>
<td>190.9 b</td>
<td>26.3 b</td>
<td>2.6 a</td>
</tr>
<tr>
<td>RT</td>
<td>2000</td>
<td>14.58 d</td>
<td>0.57 a</td>
<td>320.0 c</td>
<td>37.7 c</td>
<td>3.9 b</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>ST</td>
<td>2001</td>
<td>6.40 a</td>
<td>0.62 b</td>
<td>231.4 a</td>
<td>25.1 a</td>
<td>1.4 a</td>
</tr>
<tr>
<td>MET</td>
<td>2001</td>
<td>9.67 b</td>
<td>0.59 a</td>
<td>275.2 b</td>
<td>32.2 b</td>
<td>2.4 b</td>
</tr>
<tr>
<td>HET</td>
<td>2001</td>
<td>10.18 b</td>
<td>0.61 a</td>
<td>319.4 b</td>
<td>35.8 b</td>
<td>2.3 b</td>
</tr>
<tr>
<td>RT</td>
<td>2001</td>
<td>16.19 c</td>
<td>0.60 a</td>
<td>473.5 c</td>
<td>39.7 c</td>
<td>2.9 c</td>
</tr>
<tr>
<td></td>
<td>2001</td>
<td>+++</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

au: absorbance units
**,**,** indicate significance at P<0.05, P<0.01, P<0.001 respectively
The modified wine colour density of wines after fermentation was as follows: 7.1 and 8.0 (ST), 9.6 and 12.1 (MET), 9.6 and 11.7 (HET) and 19. and 14.4 (RT) in the first and second season respectively (Table 6.8). The calculated parameters of wine colour density and wine hue were lower for ST wines which was a result of low readings at both 520 nm and 420 nm. Higher modified wine colour density and hue for RT wines were calculated form higher readings at both 520 nm and 420 nm than those of the other treatments. Modified wine hue was significantly \((P<0.001)\) higher in ST wines (0.49 and 0.51) compared to MET (0.47 and 0.45), HET (0.46 and 0.47) and RT (0.37 and 0.47 in the first and second season respectively) (Table 6.8).

In the 2000 season the concentration of total anthocyanins (mg/L) in wines after fermentation, as determined by the UV/visible spectroscopy method (Somers and Evans 1977), was similar for ST, MET and HET (430-486 mg/L) while significantly higher \((P<0.001)\) for RT (787 mg/L) (Table 6.8). In the second season the concentration of total anthocyanins in wines after fermentation was significantly lower \((P<0.001)\) for ST (405 mg/L) compared to MET (569 mg/L), HET (554 mg/L) and RT (643 mg/L) (Table 6.8).

The concentration of total phenolics in wines after fermentation in both seasons were significantly \((P<0.001)\) lower in ST wines (29 and 29 au/L) and higher in RT wines (51 and 45 au/L) compared to MET (31 and 40 au/L) and HET (33 and 40 au/L in the first and second season respectively) (Table 6.8). The concentration of polymeric pigments in wines after fermentation was significantly \((P<0.001)\) lower in ST wines while significantly \((P<0.001)\) higher in RT wine, compared to other treatments (Table 6.8).

6.3.2.2. Chemical composition of wines after 12 months of ageing
The concentration of anthocyanins
After 12 months of ageing, the concentration of total anthocyanins in wines from the 2000 season, as determined by the HPLC method, was similar in ST, MET and HET (182-210 mg/L) while RT was significantly higher \((P<0.001)\) being 296 mg/L (Figure 6.5). The same trend was present for the concentration of anthocyanins in the monoglucoside form and the acetyl-glucoside form, while the concentration of anthocyanins in the coumaroyl-glucoside form was significantly different \((P<0.001)\) only between ST
and RT (Figure 6.5). The concentration of total anthocyanins in wines from 2001 season after 12 months of ageing was significantly \( (P<0.001) \) lower in ST (337 mg/L) and significantly \( (P<0.001) \) higher in RT (540 mg/L) than MET had 389 mg/L and HET had 421 mg/L (Figure 6.5).

In the first season after 12 months of ageing, the concentration of total anthocyanins decreased by 70% whilst in the second season the decrease was 60%. In both seasons MET showed the highest decrease in the concentration of total anthocyanins (74% and 61%) compared to HET (71% and 56%) and ST (73% and 54% in the first and second season respectively). The decrease in the concentration of anthocyanins in the acetyl glucoside form was by 74-78% and 61-67%, coumaroyl-glucoside form 70-74% and 56-60% and mono-glucoside form 69-73% and 50-57% in the first and second season respectively.

The proportions of anthocyanins in the various forms changed over twelve months of ageing (Table 6.6). All treatments showed an increase in the proportion of anthocyanins in the mono-glucoside form, while the proportion of anthocyanins in the coumaroyl-glucoside and acetyl glucoside form decreased (Table 6.6).

The concentration of total flavan-3-ols and flavonols
During 12 months of ageing the concentration of total monomeric flavan-3-ols (catechin, epicatechin and epicatechin gallate) decreased by 20-45% and was as follows: 20, 23, 26 and 18 mg/L in wines of ST, MET, HET and RT in the first season respectively. In the second season the decrease of 50% was as follows: 20, 30, 30 and 19 mg/L in wines of ST, MET, HET and RT respectively (Figure 6.6).

After 12 months of ageing the concentration of quercetin-3-glucoside in wines was 4.1-4.9 mg/L and 3.3-4.5 mg/L, which represented a decrease of 65-80% and 50-60%, in the first and second season respectively (Figure 6.7). The concentration of quercetin, which decreased by more than 80%, was as follows: 0.1, 0.4, 0.6 and 0.5 and 0.1, 0.3, 0.3 and 0.4 mg/L in wines of ST, MET, HET and RT in the first and second season respectively (Figure 6.7).
The concentration of polymeric pigments and tannins

The concentration of polymeric pigments (expressed as mg M3G equivalents/L) in wines after 12 months of ageing, as determined by the HPLC method, was significantly\((P<0.001)\) lower in ST \((108 \text{ and } 107)\) and higher in RT \((208 \text{ and } 255)\) while MET had \(170 \text{ and } 174\) and HET had \(125 \text{ and } 159\) mg/L in the first and second season respectively (Figure 6.8). The increase of polymeric pigment in wines after 12 months of ageing was higher in the first season \((63\%, \ 54\%, \ 52\% \text{ and } 51\% \text{ for MET, HET, ST and RT respectively})\) than in the second season \((36\%, \ 34\%, \ 32\% \text{ and } 31\% \text{ for ST, HET, MET and RT respectively})\) (Figure 6.8).

The concentration of tannins (express as mg catechin equivalents/L) was significantly\((P<0.001)\) lower in ST wines \((434 \text{ and } 304)\) and significantly higher in RT wines \((801 \text{ and } 917)\) than the other treatments (Figure 6.8). In both season MET had higher concentration of tannins \((561 \text{ and } 652)\) than HET \((480 \text{ and } 646\) mg/L). When comparing between seasons, in the second season the concentration of tannins in wines was lower \(22\% \text{ for ST but higher } 14\% \text{ for MET, } 25\% \text{ for HET and } 13 \% \text{ for RT (Figure 6.9).}\)

Modified wine colour density and modified wine hue

The differences in the modified wine colour density of wines after 12 months of ageing was similar to those of wines after fermentation. The only exception was that MET \((2000)\) which was significantly different \((P<0.001)\) to HET \((2000)\) which was due to higher values of both absorbance at 520 nm \((15\%)\) and 420 nm \((10\%)\) (Table 6.8). Modified wine hue of wines after 12 months of ageing was higher for ST and HET then MET and RT in the first season, but in the second season only ST was higher than other treatments (Table 6.8).

The concentration of total anthocyanins in wines after 12 months of ageing, as determined by the UV/visible spectroscopy method, was significantly lower \((P<0.001)\) for MET in the first and for ST in the second season, while RT was significantly higher \((P<0.001)\) than other treatments in both seasons (Table 6.8). The decrease in the concentration of total anthocyanins in wines after 12 months of ageing was the same for ST, HET and RT \((40 \text{ and } 58\%)\) while in wines of MET was \(27 \text{ and } 48\%\) in the first and second season respectively.
The concentration of polymeric pigments in wines after 12 months of ageing followed the same trend as in wines after fermentation with MET and RT significantly higher ($P<0.001$) than ST and HET in the first season, while in the second season ST was significantly lower ($P<0.001$) and RT significantly higher ($P<0.001$) than other treatments (Table 6.8). The increase in the concentration of polymeric pigments during 12 months of ageing was 40-55% in the first and 26-39% in the second season.

The chemical composition of wines after 12 months of ageing

Wine pH, TA and VA (Table 6.9) were similar in ST, MET, HET and RT wines in both seasons. Wines of ST had a higher percentage of alcohol than other treatments while sugar and malic acid varied between treatments and seasons (Table 6.9).

Table 6.9. The chemical composition of wines after 12 months of ageing. Wines were made from berries of the following treatments: ST, MET, HET and RT in the 2000 and 2001 season. Each value represents the mean of 3 replicates. Means in columns followed by different letters are significantly different within one season.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Year</th>
<th>Wine pH</th>
<th>TA g/L</th>
<th>VA g/L</th>
<th>Free SO$_2$ mg/L</th>
<th>Total SO$_2$ mg/L</th>
<th>Alcohol %</th>
<th>Reducing sugars g/L</th>
<th>Malic Acid g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST</td>
<td>2000</td>
<td>3.29</td>
<td>6.7</td>
<td>0.36</td>
<td>4.7</td>
<td>24.3 b</td>
<td>14.1 b</td>
<td>0.27 b</td>
<td>2.24 b</td>
</tr>
<tr>
<td>MET</td>
<td>2000</td>
<td>3.38</td>
<td>6.5</td>
<td>0.32</td>
<td>3.3</td>
<td>23.3 a</td>
<td>13.3 a</td>
<td>0.10 a</td>
<td>1.48 ab</td>
</tr>
<tr>
<td>HET</td>
<td>2000</td>
<td>3.32</td>
<td>7.2</td>
<td>0.30</td>
<td>4.0</td>
<td>21.7 a</td>
<td>13.5 a</td>
<td>0.33 b</td>
<td>2.08 ab</td>
</tr>
<tr>
<td>RT</td>
<td>2000</td>
<td>3.40</td>
<td>6.5</td>
<td>0.29</td>
<td>3.0</td>
<td>21.0 a</td>
<td>13.7 a</td>
<td>0.40 c</td>
<td>1.26 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST</td>
<td>2001</td>
<td>3.43</td>
<td>7.2</td>
<td>0.37</td>
<td>12.5</td>
<td>41.7</td>
<td>15.0 c</td>
<td>0.40</td>
<td>2.01 b</td>
</tr>
<tr>
<td>MET</td>
<td>2001</td>
<td>3.30</td>
<td>6.5</td>
<td>0.31</td>
<td>13.6</td>
<td>41.1</td>
<td>14.8 b</td>
<td>0.40</td>
<td>1.75 b</td>
</tr>
<tr>
<td>HET</td>
<td>2001</td>
<td>3.43</td>
<td>6.4</td>
<td>0.35</td>
<td>14.1</td>
<td>42.4</td>
<td>14.8 b</td>
<td>0.40</td>
<td>1.37 a</td>
</tr>
<tr>
<td>RT</td>
<td>2001</td>
<td>3.36</td>
<td>6.7</td>
<td>0.31</td>
<td>13.1</td>
<td>34.4</td>
<td>14.2 a</td>
<td>0.40</td>
<td>1.33 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST</td>
<td>2001</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>MET</td>
<td>2001</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>HET</td>
<td>2001</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>RT</td>
<td>2001</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

ns not significant, *, **, *** indicate significance at $P<0.05$, $P<0.01$, $P<0.001$ respectively
6.3.3. Relationships between the phenolic composition of berries and the phenolic composition of wines

The concentration of phenolic compounds in the berry skins and seeds was plotted against the concentration of phenolic compounds in wines, modified wine density and modified wine hue.

Table 6.10. The correlation coefficients (r) of the relationships between the concentration of anthocyanins in the berry skin, as determined by the HPLC method and the UV/visible spectroscopy method, and modified wine colour density, modified wine hue, concentration of total anthocyanins, phenolics, polymeric pigments and polymeric tannins in wines after fermentation and after 12 months of ageing.

<table>
<thead>
<tr>
<th></th>
<th>Concentration of total anthocyanins in the berry skin (mg/g berry weight) as determined by HPLC</th>
<th>Concentration of total anthocyanins in the berry skin (mg/g berry weight) as determined by UV/visible spectroscopy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>After fermentation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modified wine colour density</td>
<td>0.93***</td>
<td>0.88***</td>
</tr>
<tr>
<td>Modified wine hue</td>
<td>-0.64***</td>
<td>-0.45*</td>
</tr>
<tr>
<td>Concentration of total</td>
<td>0.85***</td>
<td>0.91***</td>
</tr>
<tr>
<td>anthocyanins (mg M3G eq/L)⁴</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration of total</td>
<td>0.94***</td>
<td>0.87***</td>
</tr>
<tr>
<td>anthocyanins (mg/L)⁵</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration of polymeric</td>
<td>0.79***</td>
<td>0.91***</td>
</tr>
<tr>
<td>pigments (mg M3G eq/L)⁶</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration of polymeric</td>
<td>0.89***</td>
<td>0.82***</td>
</tr>
<tr>
<td>pigments (au units/L)⁷</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration of tannins</td>
<td>0.80***</td>
<td>0.87***</td>
</tr>
<tr>
<td>(mg cat eq/L)⁸</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>After 12 months of ageing</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modified wine colour density</td>
<td>0.94***</td>
<td>0.88***</td>
</tr>
<tr>
<td>Modified wine hue</td>
<td>-0.37</td>
<td>-0.24</td>
</tr>
<tr>
<td>Concentration of total</td>
<td>0.70***</td>
<td>0.83***</td>
</tr>
<tr>
<td>anthocyanins (mg M3G eq/L)⁴</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration of total</td>
<td>0.78***</td>
<td>0.86***</td>
</tr>
<tr>
<td>anthocyanins (mg/L)⁵</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration of polymeric</td>
<td>0.79***</td>
<td>0.91***</td>
</tr>
<tr>
<td>pigments (mg M3G eq/L)⁶</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration of polymeric</td>
<td>0.73***</td>
<td>0.61**</td>
</tr>
<tr>
<td>pigments (au units/L)⁷</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration of tannins</td>
<td>0.97***</td>
<td>0.89***</td>
</tr>
<tr>
<td>(mg cat eq/L)⁸</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

au: absorbance units
⁴ as determined by the HPLC method
⁵ as determined by the spectrophotometric method of Somers and Evans (1977)
*, **, *** indicate significance at P<0.05, P<0.01, P<0.001 respectively
There were highly significant ($P<0.001$) positive correlations between the concentration of total anthocyanins in the berry skin, as determined by the HPLC or UV/visible spectroscopy method, and i) modified wine colour density (the correlation coefficient ($r$) was 0.88-0.94), ii) concentration of total anthocyanins ($r=0.70-0.94$) and iii) polymeric pigments ($r=0.61-0.94$) and iv) tannins ($r=0.80-0.87$), in both wines after fermentation and after 12 months of ageing (Table 6.10).

Table 6.11. The correlation coefficients ($r$) of the relationships between the concentration of phenolics in the berry as determined by the HPLC method and UV/visible spectrophotometry and the wine colour density, wine hue, concentration of total anthocyanins, phenolics, polymeric pigments and polymeric tannins in wines after fermentation and after 12 months of ageing.

<table>
<thead>
<tr>
<th></th>
<th>Concentration of skin tannins determined by HPLC (mg cat eq/g berry weight)</th>
<th>Concentration of skin phenolics determined by UV visible spectrophotometry (au/g berry weight)</th>
<th>Seed tannins (mg cat eq/g berry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>After fermentation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modified wine colour density</td>
<td>0.89***</td>
<td>0.88***</td>
<td>-0.36</td>
</tr>
<tr>
<td>Modified wine hue</td>
<td>-0.48*</td>
<td>-0.45*</td>
<td>0.11</td>
</tr>
<tr>
<td>Concentration of tannins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mg cat eq/L)</td>
<td>0.93***</td>
<td>0.82***</td>
<td>-0.52**</td>
</tr>
<tr>
<td>Concentration of total phenolics (au units/L)</td>
<td>0.91***</td>
<td>0.89***</td>
<td>-0.44*</td>
</tr>
<tr>
<td>Concentration of polymeric pigments (mg M3Geq/L)</td>
<td>0.94***</td>
<td>0.86***</td>
<td>-0.50*</td>
</tr>
<tr>
<td>Concentration of polymeric pigments (au units/L)</td>
<td>0.85***</td>
<td>0.84***</td>
<td>-0.25</td>
</tr>
<tr>
<td><strong>After 12 months of ageing</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modified wine colour density</td>
<td>0.91***</td>
<td>0.88***</td>
<td>-0.34</td>
</tr>
<tr>
<td>Modified wine hue</td>
<td>-0.41</td>
<td>-0.23</td>
<td>0.01</td>
</tr>
<tr>
<td>Concentration of tannins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mg cat eq/L)</td>
<td>0.94***</td>
<td>0.87***</td>
<td>-0.37</td>
</tr>
<tr>
<td>Concentration of total phenolics (au units/L)</td>
<td>0.94***</td>
<td>0.92***</td>
<td>-0.48*</td>
</tr>
<tr>
<td>Concentration of polymeric pigments (mg M3G eq/L)</td>
<td>0.94***</td>
<td>0.86***</td>
<td>-0.50*</td>
</tr>
<tr>
<td>Concentration of polymeric pigments (au units/L)</td>
<td>0.66***</td>
<td>0.61***</td>
<td>-0.06</td>
</tr>
</tbody>
</table>

au: absorbance units  
* as determined by the HPLC method  
* as determined by the spectrophotometric method of Somers and Evans (1977)  
* * * * * indicate significance at $P<0.05$, $P<0.01$, $P<0.001$ respectively
There were highly significant positive correlations between the concentration of skin tannins as determined by the HPLC method and skin phenolics as determined by the UV/visible spectroscopy method and i) wine colour density (r=0.88-0.91) ii) the concentration of tannins (r=0.82 and 0.94), iii) total wine phenolics (r=0.89-0.94), iv) the concentration of polymeric pigments (as determined by the HPLC method) (r=0.86 and 0.94) and v) the concentration of polymeric pigments (as determined by the UV/visible spectroscopy) (r=0.61 and 0.85) in both wines after fermentation and after 12 months of ageing (Table 6.11).

There were negative correlations between the concentration of seed tannins and i) the concentration of tannins (as determined by the HPLC method) (r=-0.52), ii) the concentration of total wine phenolics (as determined by the UV/visible spectroscopy method) (r = -0.44) and iii) the concentration of polymeric pigments (as determined by the HPLC method) (r = -0.50) (Table 6.11). These relationships were less prominent in wines after 12 months of ageing (Table 6.11).

Furthermore relationships between modified wine colour density and phenolic compounds in wines were investigated (Table 6.12). The value of the correlation coefficient (r) indicated that modified colour density was strongly correlated with the concentration of anthocyanins in wines after fermentation (0.91 and 0.98) and in wines after 12 months of ageing (0.55 and 0.75, as determined by the HPLC method and UV/visible spectroscopy method respectively) (Table 6.12). Modified wine colour density was also highly correlated with the concentration of polymeric pigments in wines after fermentation, (r=0.83 and 0.96) and in wines after 12 months of ageing (r=0.95 and 0.85, as determined by the HPLC method and UV/visible spectroscopy method).

The value of the correlation coefficient (r) indicated that the relationship between the concentration of total anthocyanins and polymeric pigments was slightly stronger in wines after fermentation (r=0.85 and 0.94, P<0.001) than in wines after 12 months of ageing (r=0.61 and 0.91, P<0.001). The increase in the concentration of polymeric pigments in wines after 12 months of ageing was strongly correlated with the decrease in the concentration of total anthocyanins (r=0.84 and 0.72, P<0.001, as determined by the HPLC method and by UV/visible spectroscopy method).
Table 6.12. The correlation coefficients ($r$) of the relationships between modified wine colour density and the concentration of anthocyanins, phenolics, polymeric pigments and tannins in wines after fermentation and after 12 months of ageing, as determined by the HPLC method and the UV/visible spectroscopy method.

<table>
<thead>
<tr>
<th>After fermentation</th>
<th>Modified wine colour density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration of total anthocyanins (mg M3G eq/L)$^a$</td>
<td>0.91***</td>
</tr>
<tr>
<td>Concentration of total anthocyanins (mg/L)$^b$</td>
<td>0.98***</td>
</tr>
<tr>
<td>Concentration of polymeric pigments (mg M3G eq/L)$^a$</td>
<td>0.83***</td>
</tr>
<tr>
<td>Concentration of polymeric pigments (au units/L)$^b$</td>
<td>0.96***</td>
</tr>
<tr>
<td>Concentration of tannins (mg cat eq/L)$^a$</td>
<td>0.89***</td>
</tr>
<tr>
<td>Concentration of total phenolics (au units/L)$^b$</td>
<td>0.98***</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>After 12 months of ageing</th>
<th>Modified wine colour density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration of total anthocyanins (mg M3G eq/L)$^a$</td>
<td>0.55**</td>
</tr>
<tr>
<td>Concentration of total anthocyanins (mg/L)$^b$</td>
<td>0.75***</td>
</tr>
<tr>
<td>Concentration of polymeric pigments (mg M3G eq/L)$^a$</td>
<td>0.96***</td>
</tr>
<tr>
<td>Concentration of polymeric pigments (au units/L)$^b$</td>
<td>0.85***</td>
</tr>
<tr>
<td>Concentration of tannins (mg cat eq/L)$^a$</td>
<td>0.93***</td>
</tr>
<tr>
<td>Concentration of total phenolics (au units/L)$^b$</td>
<td>0.91***</td>
</tr>
</tbody>
</table>

$^a$ : absorbance units
$^b$ : as determined by the HPLC method

6.3.4. Sensory evaluation of wines after 12 months of ageing

Descriptive sensory analysis

Mouth-feel attributes (Table 6.3) were rated in wines from the first (2000) and second vintage (2001). Attributes such as silky, velvety, coarse, grainy and drying were rated in mouth (im) and after expectoration (ae).

The wines from the 2000 vintage were significantly different in coarseness, dryness, puckery, adhesiveness and overall astringency, as well as in overall fruit flavour. The
wines from the extensive shading treatment were rated slightly higher for fullness and silkiness than wines of the other treatments. The wines from the RT treatment rated higher for the most of the attributes (coarseness, dryness, puckery, adhesiveness, astringency and fruit flavour) (Figure 6.11). For the same attributes the wines of the HET treatment was rated higher than wines from the MET treatment.

Figure 6.11. Mean ratings for mouth-feel attributes in wines from the 2000 vintage. Wines were made from berries of the following treatments: ST (1-10% sunlight at the bunch zone), MET (10-50% sunlight at the bunch zone), HET (40-80% sunlight at the bunch zone) and RT. Each value is the mean score from two fermentation replicate wines that were presented to 14 judges in three replicate sessions. LSD is the least significant difference. *, **, *** indicate significance at P<0.05, P<0.01, P<0.001 respectively.

The differences in the mouth-feel attributes in wines from the 2001 vintage were more prominent and the wines were significantly different (P<0.001) in surface smoothness (silkiness, coarse/emery, grainy), adhesiveness, dryness, astringency persistence, overall fruit flavour and fruit flavour persistence (Figure 6.12). Additionally, the wines differed in hotness and fullness (P<0.001). The wines of the ST treatment were rated higher for silkiness, fullness and hotness, but lower for the most of other attributes than the wines from other treatments (Figure 6.12). There was no significant difference in the ratings of
the wines from the RT and HET treatments, while the wines from the MET treatment were rated lower for coarseness ($P<0.001$) and dryness ($P<0.05$) (Figure 6.12).

In both seasons, the astringency-related mouth-feel attributes were highly correlated among themselves, but inversely to silkiness. Another interesting finding was that hotness and fullness were positively correlated with silkiness ($r=0.73$), but negatively with all other astringent attributes.

Figure 6.12. Mean ratings for mouth-feel attributes in wines from the 2001 vintage. Wines were made from berries of the following treatments: ST (1-10% sunlight at the bunch zone), MET (10-50% sunlight at the bunch zone), HET (40-80% sunlight at the bunch zone) and RT. Each value is the mean score from three fermentation replicate wines that were presented to 13 judges in three replicate sessions. LSD is the least significant difference. ***, ** indicate significance at $P<0.05$, $P<0.01$, $P<0.001$ respectively.

Wine aroma attributes significantly differed between treatments (Figure 6.13). The wines from the ST treatment were rated significantly higher ($P<0.001$) for herbaceous and straw ($P<0.01$) aroma, but lower for plum and liquorice ($P<0.01$) (Figure 6.13). The wines form MET and HET treatments were rated similarly for all aroma attributes.
except that herbaceous aroma was more prominent in MET wines. Liquorice aroma was more present in the wines of the RT treatment than other wines while straw aroma was rated higher in RT wines than in wines of MET and HET. Raspberry and cherry aroma was rated similarly in all wines (Figure 6.13).

![Graph showing aroma ratings for different treatments](image)

Figure 6.13. Mean ratings for aroma attributes in wines from the 2001 vintage. Wines were made from berries of the following treatments: ST (1-10% sunlight at the bunch zone), MET (10-50% sunlight at the bunch zone), HET (40-80% sunlight at the bunch zone) and RT. Each value is the mean score from three fermentation replicate wines that were presented to 13 judges in three replicate sessions. LSD is the least significant difference *,**,*** indicate significance at $P<0.05, P<0.01, P<0.001$ respectively.

Principal component analysis was performed for nine of the attributes (the mean values) rated for wines from both vintages in attempt to group samples accordingly to their sensory properties (Figure 6.14). The first component explaining 71% of total variance was associated with astringency related attributes (puckery and adhesiveness), while the second component which explained 26% of total variance was related to silkiness and fullness (Figure 6.14). Wines of the ST treatment (ST 01) were clearly separated on a basis of perceived surface smoothness (silkiness), while wines of the HET and RT
treatments were rated as wines with rougher texture than wines of the MET treatment (Figure 6.14).

Figure 6.14. The principal component analysis for the mean sensory profile of wines from the (00) 2000 and (01) 2001 vintage. Wines were made from berries of the following treatments: ST (1-10% sunlight at the bunch zone), MET (10-50% sunlight at the bunch zone), HET (40-80% sunlight at the bunch zone) and RT. in the 2000 and 2001 season. Each value for wines from the 2000 vintage is the mean score from two fermentation replicate wines that were presented to 14 judges in three replicate sessions. Each value for wines from the 2001 vintage is the mean score from three fermentation replicate wines that were presented to 13 judges in three replicate sessions.

**Quality rating and preference test**

Series of evaluations were carried with a panel of experienced tasters asked to score the wines using a 20-point quality scale, which is commonly applied in the Australian wine industry. The wines from the ST treatments were rated lower in both seasons, while the wines of the RT treatment were rated significantly higher ($P<0.001$) than other treatments. There was no significant difference in the rating of MET and HET wines (Table 6.13).
Table 6.13. The mean score (± standard error of the mean) on a 20-point scale of wines after 12 months of ageing from the 2000 and 2001 vintage. Each value represents the mean of 2 (2000 vintage) or 3 (2001) replicates. Means in columns followed by different letters are significantly different within the same season.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Vintage</th>
<th>Quality score</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST</td>
<td>2000</td>
<td>14.2±0.12 a</td>
</tr>
<tr>
<td>MET</td>
<td>2000</td>
<td>14.8±0.10 b</td>
</tr>
<tr>
<td>HET</td>
<td>2000</td>
<td>14.8±0.01 b</td>
</tr>
<tr>
<td>RT</td>
<td>2000</td>
<td>15.8±0.05 b</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>***</td>
</tr>
<tr>
<td>ST</td>
<td>2001</td>
<td>14.5±0.20 a</td>
</tr>
<tr>
<td>MET</td>
<td>2001</td>
<td>15.9±0.20 b</td>
</tr>
<tr>
<td>HET</td>
<td>2001</td>
<td>15.4±0.18 b</td>
</tr>
<tr>
<td>RT</td>
<td>2001</td>
<td>16.6±0.24 c</td>
</tr>
<tr>
<td></td>
<td>2001</td>
<td>***</td>
</tr>
</tbody>
</table>

*** indicate significance at $P<0.001$

The panellists were also asked to rank the wines according to their personal preference. In both seasons, although there was no significant difference ($P<0.05$) in ranking, there was a trend that preferred wines were the RT wines, followed by the wines of HET, MET and ST.

**Colour assessment**

The assessment of red wine colour and purple hue was carried out on wines from the second vintage. The results showed that the difference in red colour and purple hue can be made visually. Clearly both red colour and purple hue colours were rated significantly lower ($P<0.001$) in wines of the ST treatment and significantly higher in wines of the RT treatment than in the wines from the other treatments (Figure 6.15).
6.4. Discussion

6.4.1. Phenolic compounds in wines

6.4.1.1. Anthocyanins
The concentration of total anthocyanins in wines after fermentation (as determined by HPLC) was 670-1045 mg/L and 740-1340 mg/L in the first and second season respectively (Figure 6.5). Malvidin and its derivatives represented 85%, peonidin and peonidin 5-7%, delphinidin 2% and cyanidin less than 1%. The proportion of anthocyanins in the mono-glucosides, acetyl-glucosides and coumaroyl-glucosides form in wines (60%, 30% and 10%) varied to that in berry skins (40%, 20% and 40%). A higher proportion of mono-glucosides and lower proportion of coumaroyl-glucosides in
wines compared to skins were also observed in berries and wines of 5 cultivars in France (Roggero et al. 1984), 3 cultivars in Italy (Leone et al. 1984), 14 cultivars in Portugal (Bakker and Timberlake 1985b) and 3 cultivars in Uruguay (Gonzalez-Neves et al. 2001). Bakker and Timberlake (1985a) indicated that a low concentration of ethanol (approximately 14%) produced during fermentation would extract a smaller amount of malvidin-coumaroyl glucoside from skins, than 50-85% methanol, which is usually used for the extraction of anthocyanins from skins. This may explain the decreased proportion of malvidin coumaroyl glucoside, but does not explain the increased proportion of malvidin mono-glucoside. The differences could be attributed to hydrolysis of malvidin coumaroyl-glucoside during fermentation and during wine ageing, which would result not only in a decrease of malvidin coumaroyl-glucoside, but also in an increase of malvidin mono-glucoside. Leone et al. (1984) observed that within 24 h from crushing of the grapes, the proportion of malvidin coumaroyl-glucoside decreased from 27.6% to 12.3% and after 2 and 7 months to 12% and 9.9% respectively. At the same time, malvidin mono-glucoside increased from 44.4%, through 43%, to 46.3% while malvidin acetyl-glucoside changed from 20.9% to 23.7% and finally to 29%. The hypothesis that hydrolysis of acylated anthocyanins can lead to non-acylated anthocyanins has been further supported by the study of the condensation reaction between malvidin acetyl-glucoside, catechin and acetaldehyde in model wine solution when some purple pigments derived from malvidin acetyl-glucoside as well as some pigments derived from malvidin mono-glucoside were formed (Pazo-Alvarez et al. 1997).

During 12 months of ageing, in both seasons, the concentration of anthocyanins in the acetyl-glucoside form showed the largest reduction (75-78% and 61-68%), followed by anthocyanins in the coumaroyl-glucoside form (70-74% and 56-62%) and anthocyanins in the mono-glucoside form (69-72% and 50-57% in the first and second season respectively). The results indicated that the degradation of anthocyanins acylated with acetic acid was greater than those acylated with p-coumaric acid which agrees with other studies performed in model solutions (Romero and Bakker 1999) or in wine (Dallas et al. 1995, Mateus et al. 2001), but contrasts with the findings of McCloskey and Yengoyan (1981). Although it appears that during wine ageing degradation of non-acylated anthocyanins was slower, it could be due to hydrolysis of acylated anthocyanins which then might compensate for the loss of mono-glucosides.
6.4.1.2. Flavan-3-ols and flavonols in wines

It is believed that monomeric and polymeric phenolic compounds are more easily extracted from skins than from seeds; this appears to be related to their localisation in grape tissues and various linkages between phenolic and other compounds, such as proteins or polysaccharides (Singleton and Draper 1966, Singleton and Esau 1969, Meyer and Hernandez 1970, Amrani Joutei et al. 1994, Prieur et al. 1994, Thorngate and Singleton 1994, Sun et al. 1999). Sun et al. (1999) reported that during fermentation 45-50% of monomers and oligomers from seeds and nearly all monomers, oligomers and polymeric tannins from skins were extracted into wines. Although the extractability of flavan-3-ol monomers from seeds could be lower than from skins, their concentration in seeds (0.10-0.38 mg/g berry weight) was ten-fold higher than in skins (0.010-0.024 mg/g berry weight). Thus it was likely that a greater proportion of flavan-3-ol monomers in wines (0.35-0.65 mg/L) originate from seeds. However, during 12 months of ageing the concentration of total monomeric flavan-3-ols in wines of all treatments decreased by 40-50% to (20-30 mg/L) with the highest decrease observed in wines of the ST treatment, although the reason for this remains uncertain.

The difference in the concentration of quercetin-3-glucoside and quercetin in the berry skin between different treatments (0.02-0.13 mg/g berry weight) was greater than in wines after fermentation (12-22 mg/L and 8-11 mg/L in the first and second season respectively) (Figure 6.7). During 12 months of ageing in wines of all treatments the concentration of total flavonols (sum of quercetin and quercetin-3-glucoside) decreased by 60-70% to 3-5 mg/L (Figure 6.7). Interestingly, after 12 months of ageing, lower concentration of flavonols was determined in wines that were made from berries with higher concentration of flavonols (RT wines). The study of copigmentation has shown that quercetin may act as a strong copigment (Lambert 2002). That role of quercetin in wines remains uncertain because the concentrations used in the copigmentation studies were much greater than those determined in wines. Furthermore, Price et al. (1995a) hypothesised that higher level of quercetin-3 glucoside may confer colour stability, but the critical level that would have an effect on wine colour requires further research.
6.4.1.3. Pigmented and non-pigmented polymers

The mechanisms considered responsible for the formation of polymeric and non-polymeric pigments include copigmentation, self-association and acetaldehyde-mediated condensation. Many studies have shown that an increase in polymerised level of tannins and the copolymerisation between anthocyanins and tannins depends on their concentration and composition (Baranowski et al. 1983, Bakker et al. 1993, Picinelli et al. 1994, Dallas et al. 1996b, Cheynier et al. 2000, Romero and Bakker 2000). Thus it was expected that the formation of pigmented polymers would be greater in wines that were made from berries with a higher concentration of both anthocyanins and tannins. That was generally true for the concentration of polymeric pigments in wines after fermentation. When wines of the ST, MET and HET treatment in the 2000 vintage were made from berries with similar concentrations of anthocyanins and tannins, the concentration of polymeric pigments in wines of these treatments after fermentation was similar as well. In the second season, the concentration of polymeric pigments was significantly lower ($P<0.001$) in wines of ST and significantly higher in wines of RT than the other treatments (Figure 6.8) which was again a reflection of the differences observed in berries.

After 12 months of wine ageing the differences in the concentration of polymeric pigments between treatments were more pronounced than in wines after fermentation, particularly in wines of the ST and HET treatment from the 2000 vintage. Another interesting observation was that the concentration of polymeric pigments in wines of the ST and MET treatment reached similar level in both seasons. The concentration of polymeric pigments in wines of the RT and HET treatment was greater in the second than in the first season. The concentration of polymeric pigments in wines after 12 months of ageing, although not solely attributed to the loss of anthocyanins, was highly correlated ($P<0.001$) with degradation of total anthocyanins ($r=0.82$), anthocyanins in the mono-glucoside form ($r=0.89$), anthocyanins in the acetyl-glucoside form ($r=0.88$) and anthocyanins in the coumaroyl-glucoside form ($r=0.63$). These data raised the question whether formation of pigmented polymers was more related to the composition of anthocyanins (eg. if mono-glucosides were more likely to be incorporated into polymers than coumarates) or to the concentration of anthocyanins (including a balance between the anthocyanins and tannins concentration in berry skin) or both?
The copigmentation study of Lambert (2002) has shown that malvidin coumaroyl-
glucoside was involved in intramolecular copigmentation and did not form self-
associated aggregates. Malvidin mono-glucoside had a preference to form self-
associated aggregates over intermolecular copigmentation, but it was indicated that
during wine ageing, intermolecular copigmentation may be more pronounced due to the
greater concentration of anthocyanins relative to copigments. However, the study also
showed that the greater the degree of copigmentation in wines after fermentation, the
more polymeric pigments would be in wines after 12 months of ageing.

Many studies agree that higher concentrations of anthocyanins and tannins would lead
to higher concentration of pigmented polymers. Singleton and Trousdale (1992)
suggested that there was an optimal ratio between tannins and anthocyanins (T/A) for
incorporating pigments into the polymer. The proportion of pigment in the polymer was
the highest when seed tannin concentration was low relative to anthocyanins and the
polymer increase was higher after tannin addition than anthocyanin addition. An
addition of seed tannin resulted in a higher ratio of Tseed/A and increased wine hue,
while an addition of anthocyanins resulted in a lower ratio of Tseed/A and increased
wine colour density.

The ratio Tseed/A determined in this study (Table 6.5) supported the above hypothesis
in general, at least, for wines after fermentation. When wines were made from berries
with similar concentration of anthocyanins and seed tannins (ST, MET and HET in the
first season) the incorporation of these compounds into polymeric pigments was similar
in wines after fermentation. The higher level of polymeric pigments was determined in
wines made from berries with a lower ratio of seed tannin to anthocyanins (RT) while
the lower level of polymeric pigments in wines of ST in the second season may be
attributed to higher ratio of seed tannin to anthocyanins. The effect of seed tannins on
the phenolic composition of wines was minimised in this experiment, because all the
treatments had similar concentration of seed tannins, the data indicated that a lower
ratio of seed tannins to anthocyanins was preferable. Other studies also indicated that a
lower ratio of seed tannins to anthocyanins may be related to higher quality of wines
(Brossaud et al. 1998, Cheynier et al. 1998). However the ratio Tseed/A may be
insufficient to explain the differences in the level of pigmented and non-pigmented
polymers in wines, made from berries with different phenolic composition, as it does not allow for the putative effects of copigmentation and tannins originated from skins. The ratio between skin tannins and anthocyanins (Tskin/A) was also investigated (Table 6.5). The results indicated that the RT treatments had the highest concentration of skin tannins per unit of anthocyanins and the ST treatment had the lowest. The greater availability of skin tannins may result in a higher incorporation of anthocyanins to form pigmented polymers of greater stability during fermentation and wine ageing. A lower concentration of skin tannins relative to anthocyanins (ST) would result in a smaller quantity of anthocyanins combining with tannins, while the rest of anthocyanins would be decomposed, which would also lead to lower wine colour. The ratio Tskin/A may, as well, explain the difference in the formation of polymeric pigments in wines of MET and HET, because the comparison of berry and wine composition between MET and HET treatments showed an interesting scenario. Berries from both treatments had similar concentration and composition of anthocyanins (which was related to either inhibition of degradation of anthocyanins in the skins of HET due to the high temperature effect), similar concentration of seed tannins and skin tannins, but the concentration of pigmented and non-pigmented polymer in wines was different. The difference in the level of pigmented polymers in wines between MET and HET was greater (particularly in the first season) than the difference in the level of total tannin polymer. An estimate calculation showed that in wines after fermentation the proportion of pigmented polymer relative to total tannin polymer for all treatments was 31-35% and 30-40%, while in wines after 12 months of ageing it was 45-55% and 42-54% in the first and second season respectively. The lowest proportion of pigmented polymer relative to total tannin polymer was in wines of HET. These results indicate that there might be an insufficient amount of anthocyanins in the skin of HET available for the formation of anthocyanin-tannin adducts, thus the formation of tannin-tannin adducts could be more pronounced.

The results from this study indicate that not only the higher concentrations of anthocyanins and tannins was preferable, but also there should be a balance between their contents in berries which would lead to greater formation of polymeric pigments and enhanced colour stability. A great difference in the phenolic composition of wines as well as in modified colour density of MET and RT wines may be explained by the natural potential of the RT treatment to accumulate higher level of phenolic compounds
in berries. The accumulation of phenolic compounds in berries of the ST and HET treatment was probably affected by the applied treatments. Thus a lower concentration of polymeric pigments was likely a result of an insufficient content of skin tannins for the ST treatment and anthocyanins for the HET treatment. The availability of tannins and anthocyanins to form polymeric pigments may be limited not only by the natural potential of berries (the concentration of phenolic compounds and probably their solubility), but also by an extraction equilibrium (extraction of phenolic compound is a diffusion-controlled process between berries and wines) and by a saturation level of the interactions between phenolic compounds (Boulton 2001).

### 6.4.2. Modified wine colour density and modified wine hue

Colour spectral measurements modified for the effects of pH and sulphur dioxide concentration (as described in section 6.3.2.1, Table 6.7) indicated that the wines of the ST treatment were less red and more brown in colour than the wines of the other treatments. In both seasons, modified colour density of wines after fermentation and after 12 months of ageing was significantly lower ($P<0.001$) in wines of the ST treatments and significantly higher in wines of RT than in wines of the other treatments (Table 6.8). High modified wine colour density and low modified wine hue for wines of the RT treatment can be explained by high concentrations of monomeric anthocyanins, pigmented polymers and tannins (total phenolics). While lower modified wine colour density in wines of ST treatment from the 2001 vintage was attributed to the lower concentrations of monomeric anthocyanins, pigmented polymers and tannins (total phenolics), in wines from the 2000 vintage can not be solely related to either the concentration of anthocyanins, pigmented polymers or tannins (Table 6.8). The concentration of these compounds in ST wines were similar to those in wines of one or both MET or HET treatments, but modified wine colour density was lower by 26% in wines after fermentation and by 33% wines after 12 months of ageing compared to that of MET and/or HET wines.

Although in young wines monomeric anthocyanins play a great role, some studies reported that about 50% of the young wine colour may be result of copigmentation (Boulton 2001), and up to 25% to polymeric pigments (Somers 1971, 1982). It has been shown that with increasing methoxylation and glycosylation of the anthocyanins, colour
expression due to copigmentation increased (Brouillard 1983, Davies and Mazza 1993). Boulton (2001) indicated that the colour enhancement due to copigmentation can be two to ten times than that expected from the pigment alone. The study of Lambert (2002) showed that at equivalent concentrations, malvidin coumaroyl-glucoside exhibited approximately twice the colour expression than that of malvidin mono-glucoside. Thus the colour enhancement, due to intramolecular copigmentation of malvidin coumaroyl-glucoside could play a role in colour stabilisation in young wines as long as malvidin coumaroyl-glucoside is present (Lambert 2002). At the same time, the colour enhancement due to copigmentation of malvidin mono-glucoside by self-association is a concentration dependent effect, which is important in young wines, but would diminish with malvidin mono-glucoside loss during wine ageing (Asenstorfer 2001).

Other studies have showed that in the weeks following fermentation, lower wine colour density may be attributed to the loss of monomeric anthocyanins due to their condensation reactions (Somers and Evans 1979), but sometimes the loss in colour could be a larger percentage than that of the loss of anthocyanins (Ribereau-Gayon 1982). That difference could be attributed to a break up of the copigmentation stacks formed during fermentation (Boulton 2001). Based on the data from the current and referenced studies, lower colour density in the ST wines after fermentation and after 12 months of ageing (2000 vintage) may be attributed not only to slightly reduced concentrations of pigmented polymers (not significantly different to that of HET, $P<0.05$), but also to changed anthocyanin profile.

In the first season modified wine colour density of HET wines after fermentation was similar to the modified colour density of MET wines despite the differences observed in their phenolic composition. The concentration of polymeric pigments was significantly higher in wines of MET but the wines were similar in the concentration of anthocyanins and total tannins. The difference in wine colour density between wines with similar anthocyanin contents in other studies was related to the procyanidin contents (Timberlake and Bridle 1976, Sun et al. 2001). It has been indicated that the colour of young red wine can be affected by much higher total amounts of oligomeric and polymeric procyanidins relative to that of anthocyanins, in which case yellow-brown colour of polymerised tannins would mask red colour of polymeric pigments (Ribereau-
Gayon 1982). By analogy, yellow-brown pigments could contribute to the modified colour density of HET wines after fermentation (Table 6.8).

After 12 months of ageing, the concentration of monomeric anthocyanins was lower in wines of HET, but the concentration of polymeric pigments much higher, which contributed to a higher colour density, but lower wine hue than in wines of HET. It remains uncertain if after 12 months of ageing, the concentration of polymeric pigments was exceptionally high in wines of MET or much lower, than expected, in wines of HET (Figure 6.8). As mentioned before, copigmentation and formation of polymeric pigments in wines of the HET treatment could have been reduced due to insufficient content of anthocyanins relative to skin tannins which would reduce the formation of anthocyanin-tannin polymers and wine colour density. At the same time, the formation of tannin-tannin polymers could be increased which would contribute to the wine colour density and increase wine hue. There was no indication that higher modified wine colour density was due to oxidation of MET wines from the 2000 vintage, despite a low level of free SO₂ in wines (Table 6.9), as in the case of oxidation, modified wine hue would have been expected to increase and no oxidation flavour was detected during the sensory assessment of wines.

The contribution of phenolic compounds in wines to the wine colour were further supported by highly significant ($P<0.001$) correlations established between modified wine colour density and phenolic composition of wines (Table 6.12). Modified colour density determined in wines after fermentation was highly correlated with the concentration of total anthocyanins ($r=0.91$ and 0.95) (as determined by the HPLC and UV/visible spectrophotometric method respectively), pigmented polymers ($r=0.83$ and 0.96), tannins ($r=0.89$) and total phenolics ($r=0.98$). After 12 months of wine ageing, modified wine colour density was less dependent on the concentration of anthocyanins ($r=0.55$ and 0.75), but more on the concentration of pigmented polymers ($r=0.96$ and 0.85), tannins ($r=0.93$) and total phenolic ($r=0.91$). This supports the observation that the contribution of polymeric pigments on wine colour increases with wine maturation (Somers 1982).
Comparison of HPLC and Somers and Evan’s measures

The concentration of total anthocyanins and polymeric pigments was determined by HPLC and the UV/Visible spectroscopy method of Somers and Evans (1977) and the results were compared. There was a highly significant correlation (P<0.001) between the values obtained by the two methods for the concentration of anthocyanins (r=0.96) and for the concentration of polymeric pigments (r=0.87), in all the wines after fermentation and after 12 months of ageing (Figure 6.16). The correlation between total phenolics (UV/Visible spectroscopy) and tannins (HPLC) was much stronger in wines after 12 months of ageing (r=0.84) than in wines after fermentation (r=0.52) (Figure 6.16). It should be noted that although there were strong linear relationship over the whole range of values, within a group of samples of a smaller range, the relationship was not that strong (Figure 6.16 a, b, c). However, the results indicated that the colour spectral measures at 520 nm can be used as a simple measurement of the concentration of pigmented polymers, while a measurement at 280 nm (for total phenolics) may be used as a general indicator of the tannin concentration.

The difference in the values of polymeric pigments obtained by the two methods was larger than that for total anthocyanins. This may be due to the presence of oligomeric anthocyanin-derived pigments which contribute to the UV/Visible spectroscopy, but they are hardly detected in HPLC chromatograms (Vivar-Quintana et al. 2002). Anthocyanins-derived pigments (oligomers and polymers) are relatively resistant to change in pH, they are not bleached by SO2 and their colour expression at wine pH is higher that that of anthocyanins alone (Fulcrand et al. 1996, Bakker and Timberlake 1997, Asenstorfer 2001). Vivar-Quintana et al. (2002) suggested that at the wine pH, vitisin-like pigments can make a great contribution to the colour density of the young wine. The contributions of each of isolated vitisin-like pigment to the wine colour density still remains uncertain (Bakker and Timberlake 1997, Asenstorfer 2001), although Schwarz et al. (2003) reported that the contribution of only vitisin A to the wine colour density is around 5%.
Figure 6.16. The relationship between UV/visible spectroscopy method of Somers and Evans and the HPLC method for determining the concentration of (a) total anthocyanins in wines, (b) polymeric pigments in wines and total phenolics/total tannin polymers (c) in wines after fermentation and (d) after 12 months of ageing, using the data from all the treatments. Significance of the correlation coefficient determined by *** is significant at \( P<0.001 \).

This study supports the use of the set of measures by Somers and Evans (1977) modified for the pH and SO₂ effects (Iland 2001) for simple measures of modified wine density, modified wine hue, polymeric pigments and total phenolics in wines. However, during sensory assessments wines may be compared by wine colour density, without pH and sulphur dioxide adjustments. The HPLC method allows a complex analysis of anthocyanins, pigmented and non-pigmented oligomers and polymers.

6.4.3. Sensory properties of wines

Sensory properties of wines from this study were determined and assessed by three types of sensory methods: descriptive sensory analysis, quality rating and a preference test. Descriptive sensory analysis was based on a vocabulary for sensory attributes developed among judges. The analysis has provided very precise information about
sensory properties of the wines, but the wines could not be rated on a quality scale or on a preference basis. Quality evaluation using a 20-point quality scale provided useful information about perceived wine characteristics, but it was based on the previous experience, expectations and preferences of the “expert” judges. Preference test was used to indicate which sample was preferred.

**Descriptive sensory analysis**
In this study the mouth-feel attributes rated by the panellists were selected from terms generated and validated in previous studies of sensory properties of wines (Gawel et al. 1997, Gawel et al. 2000, Gawel et al. 2001, Vidal et al. 2002, Vidal et al. 2003a, b,c). The attributes were selected from the categories that represent surface smoothness (silky, velvety, emery/coarse), dynamic attributes (puckery and adhesive), particulate (grainy) and drying (Gawel et al. 2000). The additional attributes were selected that characterised acidity, weight (fullness), heat (hotness) and flavour (flavour intensity and flavour length) (Gawel et al. 2000). During the discussion sessions it was decided that bitterness was not an important perception in any of the wines. In both seasons, there was no significant difference between selected fermentation replicates. All ratings of ‘in mouth’ attributes highly corresponded to the ratings of the same attributes “-after expectoration-”.

The attributes representing surface smoothness (silky, velvety, emery/coarse) were found to be significantly different in the assessed wines. In both seasons silky attribute was rated higher but all astringency related attributes lower in the wines from the extensive shading treatment (ST) which could be due to the lower concentration of tannins. The wines of the RT treatment were found to be higher in coarse, puckery, grainy and dryness, and thus overall astringency, compared to the wines of other treatments. Wines of the HET treatment from the 2000 vintage were rated higher for all astringency related attributes than wines of the MET treatments. In the following season the wines from the two treatments were rated similarly except wines of the HET treatment were found to be more coarse and dry. Higher concentration of tannins relative to that of pigmented polymers in wines of the HET treatment from the 2000 vintage, compared to MET, treatment clearly indicated the effect of tannins of perceived astringency. An interesting finding was that hotness and fullness were positively correlated with silkiness but negatively with all other astringent attributes, thus were
rated the highest \(P<0.01\) in ST wines, followed by MET wines, HET wines and RT wines. Fullness can be also related to the alcohol content, which would explain a higher rating in wines of ST treatment. In both seasons the flavour attributes were rated significantly lower \(P<0.001\) in the ST wines and significantly higher in the RT wines than in wines of the other treatments (Figure 6.11, 6.12). In order to compare the respective effects of different treatments on the mouth-feel attributes, a PCA was performed and projection of the variables and wine samples are shown in Figure 6.14. The first component (PC1) representing 71% of total variance was defined by astringency related mouth-feel attributes while the second axis representing 26% of total variance was defined by silkiness and fullness.

The major difference between wines of different treatment was in aroma attributes. The distinct aroma in wines from the extensively shaded treatment (ST) was herbaceous (Figure 6.13), liquorice in the wines of the RT treatments, while plum, liquorice and straw were rated similarly in the MET and HET wines, with herbaceous more prominent aroma in the wines of MET. There were no significant difference \(P<0.05\) in raspberry and cherry aroma in all wines from different treatments. In the previous sensory evaluation of wines made from berries grown under different levels of sunlight intensity, some differences in aroma and flavour of wines were detected and wines from shaded treatments were usually characterised by green or grass aroma (Reynolds et al. 1986, Archer and Strauss 1989, Morrison and Noble 1990, Price et al. 1995b). Although the differences in wine composition were small, the wines from exposed fruit were scored higher for overall quality, probably due to a darker colour and more intense aroma.

Highly significant correlations \(P<0.001\) were established between all astringency related mouth-feel attributes (grainy, coarseness, adhesiveness, dryness and overall astringency) and the concentration of total tannins in wines (as determined by HPLC) and total phenolics (as determined by the UV/visible spectroscopy). Many studies have showed that that astringency depends on the concentration and composition of tannins, originated from seeds and skins, and may be influenced by the degree of polymerisation and galloylation of the tannins (Ricardo da Silva et al. 1991a, Sarni-Manchado et al. 1999a, Bacon and Rhodes 2000). At the same concentration, seed and skin tannin of Cabernet Franc were found to be equally astringent when tasted in citric acid solution
and white wine (Brossaud et al. 2001). This could be due to a higher degree of galloylation of seed tannins which could compensate for a higher degree of polymerisation of skin tannins. Later it was found that while higher degree of polymerisation may increase astringency perception, the increased galloylation increased coarseness (Vidal et al. 2003a). Vidal et al. (2002) also reported that in model wine system seed tannins were more astringent, coarse and drying than skin tannins of equivalent size.

The study also indicated possible relations between perceived astringency attributes of and the concentration of pigmented polymers. The perception of grainy and coarseness in wines was highly correlated to the concentration of total tannins (r=0.92 and 0.88), total phenolics (r=0.85 and 0.79) and pigmented polymers (r=0.84 and 0.76). Similarly, dryness was highly correlated to the concentration of total tannins (r=0.85), total phenolics (r=0.88) and pigmented polymers (r=0.84). The results from this study also indicated that total tannins, polymeric pigments and anthocyanins may contribute to the flavour perception (intensity and duration). The highest correlation was established between overall flavour intensity and polymeric pigments (r=0.89) and total anthocyanins (r=0.86) which supports the finding that anthocyanins may improve wine flavour (Singleton and Noble 1976). Longer persistence and duration of fruit flavour was related to wines with higher concentration of total tannins (r=0.82) and total phenolics (r=0.68). In other studies an anthocyanin fraction was found to be was mildly astringent, and when added to model wine solution containing seed tannins it increased astringency (Brossaud et al. 2001), chalkiness and coarseness (Vidal et al. 2002, Vidal et al. 2003a). It was indicated that the astringency associated with the assessed anthocyanin fraction was probably due to polyphenolic contaminants. Recently Vidal et al. (2003c) has shown that highly purified fractions of anthocyanin mono-glucosides and coumarates did not influence either astringency or bitterness of model wine solutions.

It appears that the concentration of total tannins had an effect on the mouth-feel attributes that characterise astringency. The results indicated that polymeric pigments and free anthocyanins may contribute to astringent sensations in wines, but this would require further investigation. Surface smoothness (silkiness) was negatively correlated with the concentrations of phenolic compounds in wines (tannins, pigmented polymers
and anthocyanins) and thus with all the astringency attributes, which were closely related among themselves.

**Quality rating**

A panel of experienced assessors evaluated the wines for overall quality using a 20-pint score system. While quality scoring is somewhat problematic as a sensory method, involving a hedonic as well as an analytic component, this data provides an indication that for this panel of experienced wine assessors the wines were considered to have different overall quality. This assessment provided information that low sunlight intensity (ST) affected sensory properties of wines and these wines had a lower score on the quality scale, although the difference was significant ($P<0.001$) only in the second season (Table 6.13). All wines were scored by 3 (out of 3) for the wine colour but the wines from shaded treatment were scored lower for both aroma (4-5 out of 7 points) and palate (flavour by mouth) (6-7 out of 10 points). In both seasons the wines of the RT treatment were rated higher.

**Preference test**

A preference test was used to provide a further indication of which sample was preferred for the panel of experienced tasters. When the “expert” panellists were asked to rank the wines by preference, in both seasons, the most preferred were the RT wines, followed by the wines of HET, MET and ST.

Different sensory assessments undertaken in this study provided information about the differences in the specific sensory attributes between wines of different treatments, which could be then related to a higher quality rating or as a more preferred wine. The results from this study indicated that a higher quality wine was related to the higher concentration of phenolic compounds in wines and higher wine colour. Higher concentration of phenolic compounds (tannins) may increase wine astringency, but overall sensory perception of wines depends not only on the concentration of phenolic compounds in wines but also on the balance between their concentrations.
Prediction of the phenolic composition in wines and wine rating based on the phenolic composition of berries

The composition of phenolic compounds in grape berries may be used to predict wine composition (Table 6.10 and Table 6.11). In general, a higher concentration of anthocyanins and tannins in berries would result in a higher concentration of phenolic compounds in young red wines and a higher wine colour density. During wine ageing wine, the concentration of monomeric anthocyanins decreases and wine colour density was primarily derived from polymeric pigments and tannins. Formation of polymeric pigments and tannins was dependent not only on the initial concentrations of anthocyanins and tannins in berries, but also on the ratios between them, thus a prediction of wine quality should be based on all these variables.

Overall wine quality was highly correlated to the concentration of phenolic compounds in wine and wine colour density. A simple visual assessment of wine red colour and purple hue (Figure 6.15) was a good indicator of wine colour density. This study further supports strong relationships between colour properties of wine, flavour intensity and/or wine quality as previously reported by Somers and Evans (1974), Ortiz et al. (1995), Iland (2001).

6.5. Conclusion

- Differences in the concentration of anthocyanins in the berry skins between treatments were reflected in the differences in the concentration of total anthocyanins in wines.
- During wine ageing degradation of anthocyanins acylated with acetic acid was greater than those acylated with p-coumaric acid. Their hydrolysis might compensate for the loss in the concentration of non-acylated anthocyanins.
- The formation of polymeric pigments was related to the concentration and composition of anthocyanins and seed and skin tannins and the ratio (balance) between them.
- Modified wine colour density was related to the concentration of polymeric pigments and anthocyanins in wines. The differences in modified wine colour density between treatments were greater than the differences in the concentration of total anthocyanins in berries between treatments.
• Reduced sunlight intensity at the bunch zone affected the phenolic composition of wines. Modified wine colour density, the concentration of total anthocyanins (in the second season), polymeric pigments and tannins and total phenolics was significantly lower in wines of ST than in wines of other treatments.

• Higher concentration of tannins in wines increased the astringency related mouth-feel attributes. The wines of the RT treatment were rated higher for astringency-related attributes while the wines of the ST treatment were rated higher for silkiness and fullness than wines of other treatments.

• The distinct aroma in wines from the extensively shaded treatment (ST) was herbaceous, in the wines of the RT treatments liquorice aroma, while plum, liquorice and straw were rated similarly in the MET and HET wines.

• In general, wines with higher concentration of phenolic compounds were rated higher for most of the mouth feel attributes as well as higher on a quality scale, using a separate panel of experienced tasters.

• In order to obtain the most detail and precise measures of the phenolic compounds in wines and their changes during fermentation and wine maturation this study supports the use of the set of measures by Somers and Evans (1977) modified for the pH and sulphur dioxide effects (Ilard 2001) and an HPLC method which allows complex analysis of anthocyanins, pigmented and non-pigmented oligomers and polymers. However wine colour density may be determined without any pH and sulphur dioxide adjustments when comparing wines during sensory assessments.
Chapter 7 – General discussion and directions for future research

7.1. The background to the study
It is widely accepted that seed and berry development occur simultaneously. Seeds may affect not only the weight and the ripening rate of berries (Winkler and Williams 1936, Olmo 1946, Coombe 1960, Scienza et al. 1978, Cawthon and Morris 1982) but also the chemical composition of berries (Scienza et al. 1978, Cawthon and Morris 1982, Umarrino and Di Stefano 1996). Thus it is likely that the sequences in berry development are linked to sequences of seed development which include developmental morphological and chemical changes in seeds. The aim of this study was to investigate seed development and the relationships with berry development in order to increase knowledge about mechanisms involved in seed and berry development and ripening.

Many studies have shown that the metabolism of phenolic compounds in grape berries, in particular anthocyanins, is affected by the intensity of sunlight received at the bunch zone (Morrison and Noble 1990, Gao and Cahoon 1994, Price et al. 1995a, Dokoozlian and Kliewer 1996, Keller and Hrazdina 1998, Mabrouk and Sinoquet 1998, Haselgrove et al. 2000). However these studies did not investigate the response of seed and skin tannins to variation in light intensity at the bunch zone. This study was proposed to improve our knowledge of tannin metabolism in seeds and skins and to determine how viticultural practices, in this case manipulation of canopy microclimate, influence the metabolism of tannins in berries and hence affect phenolic composition and sensory properties of wines made from those berries.

7.2. Experimental treatments and vine response to the applied canopy manipulations
Different levels of sunlight intensity at the bunch zone were achieved by canopy manipulation. Canopy manipulation included wrapping vine canopies in bird nets to constrain the canopy (ST) and dividing and vertical positioning shoots upwards and downwards (HET). These manipulations influenced the arrangement of leaves and shoots within the canopy and hence the canopy microclimate.
When the photosynthetic capacity of leaves was limited (ST), the vines adapted to vine shading by modifying their growth habit which enabled the production of certain amount of photo-assimilates. Reduced number of shoots and foliage area in the second season for the ST treatment indicated a tendency of these vines to attempt to naturally create more open canopies which would increase both direct and diffuse light and enhance photosynthesis capacity.

The vine response to the vertical positioning of shoots upwards and downwards (HET) was reflected in an increased growth of lateral leaf area and higher number of shoots per vine. This could be a result of high temperature effect on vegetative growth of Shiraz vines as it has been shown that high light intensity induced lateral shoot growth relative to main shoot growth not only for Shiraz vines (Buttrose 1969), but also for vines of Muscat Gordo Blanco and Ohanez (Buttrose 1968, 1969).

Vine performances, under the applied canopy modifications, were assessed using indicators of vine balance (leaf area/fruit weight and yield/pruning weight). The data showed that the balance of the vines of different treatments was similar within the same season which indicated that the difference in canopy architecture was not a limiting factor in the vine’s tendency to produce a certain level of vegetative and fruit growth. However, the applied viticultural treatments affected the architecture of the whole vine and thus the canopy microclimate. Measures of light intensity (PAR) at the bunch zone showed that the fruit of ST received less than 5% of available light (<100 PPFD), MET 10-40% of ambient (300-700 PPFD) and HET 40-80% of ambient (800-1500 PPFD). Sunlight interception would have effects on both leaf photosynthetic activity and on berry metabolism and it was not possible to separate these effects in relation to the differences in berry composition. This study justified the necessity for an assessment of canopy microclimate in conjunction with an assessment of berry composition.

7.3. Seed development and its relationships with berry development
While the pattern of berry growth followed a typical double sigmoid curve, the pattern of developmental changes of fresh seed weight and dry seed weight followed a biphasic curve (Chapter 3). Three phases in seed growth and development were defined as follows: i) a phase of seed growth, ii) an intermediate phase and iii) a phase of seed
drying and maturation. These phases were based on the developmental changes in fresh seed weight, dry seed weight and difference between fresh and dry seed weight, as well as the changes in physical appearances of seeds that include formation of seed features and seed coat colour. The rapid gain in fresh weight and increase in seed size during the first phase could be associated with cell division and differentiation (Pratt 1971). By the end of this phase, fresh seed weight reached its maximum and the colour of the seed coat changed from bright green to green-yellow. The second phase is relatively short and characterised by enlargement of the basal end of the seed which caused displacement of the chalaza towards the notch. This event could be associated with cell expansion to accumulate reserve materials (Pratt 1971). The third phase was associated with maximal gain in dry weight and a rapid decrease in fresh weight and water content. Rumination of fossettes, roughness and hardening of seed surfaces and the changes in seed colour from light brown to dark brown were distinct manifestations of a process of drying and maturation of grape seeds.

The study clearly indicated that the sequence of seed development coincided with the sequences in berry development. Maximum fresh seed weight and seed size (reached at approximately 60 DAF) coincided with the ending of the lag phase and the beginning of berry colouring while maximum dry seed weight and complete formation of seed features (at approximately 91 DAF) coincided with maximum berry weight. This study provided further evidence that seed development and berry development are related and the beginning of veraison is associated with cessation of seed growth. Another important observation was that although seed growth was completed at approximately 60 DAF, seed development and changes in the seed appearance continued until approximately 91 DAF. Further research is required to investigate seed development in berries of different varieties and in berries developed and ripened under different climatic conditions than that of this study.
7.4. Developmental changes in phenolic composition of seeds and skins

Developmental changes in the phenolic composition of seeds (flavan-3-ol monomers and tannins) occurred during particular sequences in seed and berry development. During the first phase of seed growth, the level of seed tannins increased, while the level of monomers was at minimum. The beginning and the end of the second phase of seed development (intermediate phase) was marked by the maximal level of seed tannins and maximal level of seed monomers respectively; these stages also coincided with the beginning and the end of veraison. During the third phase (a phase of seed drying and maturation) a sharp decrease in the level of total seed tannins followed the same trend as of the decline in water content. This process was completed at the point of maximum dry weight, completed development of all the seed features and coincided with the point when berries reached their maximum weight (Chapter 4).

Other studies have shown that the decline in the level of seed tannins (expressed on a per seed or berry basis) was related to their reduced extractability (Saint-Cricq de Gaulejac et al. 1997) (Kennedy et al. 2000b) which may be attributed to the formation of branched or oxidatively linked polymers (Ricardo da Silva et al. 1991a, Saint-Cricq de Gaulejac et al. 1999a, Saint-Cricq de Gaulejac et al. 1999b). It has been suggested that phenolic compounds in seeds may act as a biochemical barrier to the permeability to oxygen as their oxidation would reduce the oxygen available to the embryo (Werker 1980/81, 1997). It is also widely accepted that phenolic compounds are responsible for seed coat colour. Based on these assumptions and on developmental changes in the level of seed tannins and changes in seed morphology (hardening of seed coat and seed coat colour) from this study, a hypothesis was developed about the possible mechanism of oxidation of phenolic compounds, linking this work with other studies. Programmed cell death is a natural sequence in seed maturation during which oxidation of phenolic compounds occurs. Upon desiccation, seed coat cells produced ROS which caused PCD. Phenolic compounds scavenged ROS and during this process react in self-polymerisation reactions or in cross-linking reactions with proteins. This would lead to increased impermeability of the seed coat and seed dormancy (Chapter 4).
The additional information provided by this study was as follows:

- The strong inverse correlation between seed coat colour values and the level of extracted seed tannins suggested that seed coat colour could be used as an indicator of seed maturity. It is likely that a lower amount of tannins would be extracted from dark brown seeds in the winemaking process.

- Changes of the seed coat colour were related to the accumulation of total anthocyanins and skin total phenolics indicating that the external appearance and colour of the seed coat may be used as an additional indicator of an overall berry ripeness. However, it should be noted that at the later stages of maturity, seeds may reach a similar degree of brown colour, but there can still be differences in levels and concentration of seed and skin components and thus changes in seed colour may be only used as an indicator of overall berry maturity.

- Highly significant inverse correlations established between changes in the phenolic composition of seeds and changes in the phenolic composition of skins indicated that these changes occurred simultaneously and that they were related to berry maturity.

7.5. Influence of sunlight intensity at the bunch zone on the phenolic composition of seeds and skins

During seed and berry development, at a similar stage of maturity (°Brix), there was no difference in the concentration of flavan-3-ol monomers and tannins in seeds of berries from the different treatments (Chapter 4). The results clearly indicate that sunlight intensity at the bunch zone had no or only a small effect on the concentration, level and composition of flavan-3-ol monomers and seed tannins.

During berry development, at the same stage of maturity (°Brix), the concentrations of both total anthocyanins and skin total phenolics were lower in the skins of berries that were developed and ripened under reduced sunlight intensity (<5% of ambient) (ST). This supported the finding of numerous studies that excessive bunch shading decreases accumulation of phenolic compounds in the berry skin. There was no significant difference in the concentration of anthocyanins and skin total phenolics in skin of berries developed and ripened under moderate sunlight intensity (10-40% of ambient) (MET) and berries grown under high light intensity (40-80% of ambient) which is in

The comparison of the concentration and composition of phenolic compounds (anthocyanins and skin tannins) in the skins of berries from different treatments, when they attained the maximal concentration of total anthocyanins, provided detailed information about the effects of sunlight intensity at the bunch zone on the accumulation of these compounds. Excluded sunlight (BT) or reduced sunlight at the bunch zone (ST) decreased the accumulation of all phenolic compounds (anthocyanins, skin tannins and flavonols). Extensive shading resulted in enhanced accumulation of anthocyanins in the coumaroyl-glucoside form relative to those in the mono-glucoside form which is in agreement with the finding reported by Haselgrove et al. 2000. This study indicated that enhanced malvidin esterification may be associated with a greater synthesis of p-coumaric acid in the berry skins. The mechanism which leads to a shift in the rate of metabolism of anthocyanins towards coumaroyl-glucoside form under low sunlight conditions requires further research.

The concentration and composition of anthocyanins was similar in the skin of berries developed and ripened under moderate sunlight intensity (300-700 PPFD) and high light intensity (800-1500 PPFD) suggesting that it was possible that the temperature of berries highly exposed to sunlight (HET) exceeded the optimal temperature range and anthocyanin accumulation was inhibited in HET treatment. The optimum range of sunlight interception for maximum accumulation of anthocyanins was around 50% of ambient (700-800 PPFD). Most sensitive to the changes in light and temperature conditions were anthocyanins in the mono-glucoside form, followed by anthocyanins in the coumaroyl-glucoside form and acetyl-glucoside form. These patterns could be associated with reduced accumulation and/or degradation of anthocyanins; more information is required to separate the significance of each of these processes on anthocyanin metabolism.

The accumulation of skin tannins was enhanced with increasing sunlight intensity at the bunch zone, until approximately 80-90% of ambient (>1300 PPFD). The accumulation of quercetin and quercetin-3-glucoside was also higher at increased sunlight intensity at the bunch zone indicating that both compounds are highly dependent on light intensity.
The results from this study supported other studies that indicated that the concentration and composition of phenolic compounds in the berry skin depends on complex interactions between light and temperature effects on the berries. Strong relationships established between phenolic compounds in the berry skins indicated that the accumulation of anthocyanins, tannins and flavonols may be related processes, but their coordination has yet to be established. The study also indicated that in a warm or hot climate, a moderate degree of sunlight intensity (40-60% of ambient) is preferable for the optimal accumulation of all phenolic compounds in the berry skins.

7.6. Phenolic composition and sensory properties of wines
Anthocyanins extracted from the berry skins play an important role in the colour of young red wines. The results from this study showed that the differences in the concentration of total anthocyanins in the berry skins between treatments were reflected in the differences in the concentration of total anthocyanins in wines. The concentration of anthocyanins in wines decreased during wine ageing in the following order: anthocyanins in the acetyl-glucoside form > anthocyanins in the coumaroyl-glucoside form > anthocyanins in the mono-glucoside form. This indicated that degradation of anthocyanins acylated with acetic acid was greater than those acylated with p-coumaric acid. Hydrolysis of the acylated anthocyanins could compensate for the loss of the non-acylated anthocyanins and that would explain a lower decrease in the concentration of non-acylated anthocyanins compared to that of acylated anthocyanins.

Polymeric pigments are formed during condensation reactions of anthocyanins with other phenolic compounds. Under the same winemaking conditions (oxygen, SO₂, acetaldehyde, temperature, pH), the formation of polymeric pigments generally depends on the concentration and availability of anthocyanins and tannins to react and produce a variety of pigments from dimers to complex polymers which would lead to an increase in colour expression. The study investigated the ratios between the concentrations of anthocyanins and, seed and skin tannins in berries. Within the same season the contribution of seed tannins to phenolic composition of wines of different treatments was similar, but the results indicated that a lower concentration of seed tannins relative to anthocyanins concentration was preferable (Chapter 6). When the ratio between the concentration of anthocyanins and skin tannins was investigated, the results indicated
that the greater availability of skin tannins may result in a higher incorporation of anthocyanins to form pigmented polymers during fermentation and wine ageing. Contrary to this, a lower concentration of skin tannins relative to anthocyanins (ST) would result in a smaller quantity of anthocyanins being combined with tannins, while the rest of anthocyanins would be lost, which would lead to lower wine colour. The study also showed that it is very likely that there might be insufficient amounts of anthocyanins available for the formation of anthocyanin-tannin adducts if the actual concentration of anthocyanins was related to either inhibition or degradation of anthocyanins due to the high temperature effect (HET) (Chapter 5). Analysis of berry and wine composition of the RT treatment also indicated that the initial high concentration of phenolic compounds in berry skins would be associated with a high concentration of phenolic compounds in wines.

The sensory attributes of the experimental wines which related to perceived surface textural smoothness (silky, velvety, emery/coarse) were found to be significantly different in the assessed wines, with higher rating of silkiness in the wines of the ST treatment and higher rating of coarseness, velvety and overall astringency in the wines of the RT, MET and HET treatments (Chapter 6). In both seasons the flavour attributes (intensity and duration) were rated lower in wines of the ST treatment than in wines of the other treatments and a grassy aroma was rated highly in the wine from this treatment in the later season.

Highly significant correlations were established between all astringency related mouth-feel attributes (grainy, coarseness, adhesiveness, dryness and overall astringency) and the concentration of total tannins in wines (as determined by HPLC) and total phenolics (as determined by the UV/visible spectroscopy). It has been shown that astringency depends on the concentration and composition of tannins (Gawel 1998, Peleg et al. 1999, Sarni-Manchado et al. 1999b, Vidal et al. 2003b), while the contribution of anthocyanins and anthocyanin-tannin adducts still remain uncertain. Recently Vidal et al. (2002) reported that in model wine systems seed tannins are more astringent, coarse and drying than skin tannins of equivalent size. While a purified anthocyanin fraction alone was not astringent (Vidal et al. 2000c), when added to model wine solution containing seed tannins it may increase astringency (Brossaud et al. 2001), chalkiness and coarseness (Vidal et al. 2002, Vidal et al. 2003a). The possible contribution of
polymeric pigments and anthocyanins to sensory properties of wines requires further investigation.

The research provided further evidence that excessive canopy shade is detrimental to berry composition, wine composition and wine sensory properties. Berries from vines with excessively shaded vine canopies are likely to produce wines with lower wine colour density than might be expected from the measure of berry colour. This could be attributed to the composition of anthocyanins (higher proportion of anthocyanins in the coumaroyl-glucoside form) or to reduced formation of pigmented polymers, or both. The finding that the moderately and highly exposed bunches had a higher ratio of skin tannins to anthocyanins than extensively shaded bunches might explain why wines made from berries that developed and ripened under moderate to high sunlight intensity had higher levels of pigmented tannins and higher modified wine colour density. Further investigation of the formation of pigmented and non-pigmented polymers in wines and a continuation of sensory studies, particularly on specific mouth feel attributes, is necessary to gain greater knowledge and understanding of relationships between berry composition, wine composition and sensory properties of wine.
Appendix 1. Calculating grape seed colour

GRAPE SEED COLOUR CHART

Take a random sample of at least 20 seeds from a representative sample of berries.

**Method A**

Count and record the total number of seeds.
Turn all the seeds so that the top surface is up.
1. Match the colour of each seed with the colour chart. Record the number of seeds of each colour level.
2. For each group of seeds of the same colour, multiply the number of seeds by the corresponding colour number on the seed colour chart.
3. Add the separate values obtained in step 4 and divide by the total number of seeds to give the seed colour value for the seed tops.
4. Turn all the seeds so that the bottom surface is up and repeat steps 3 to 5.
5. Average the values obtained for the top and bottom surfaces to give the overall seed colour.
6. An option is to convert the overall seed colour to a percentage by dividing the overall seed colour by 11. (Note: our experience has shown that when grape berries reach maturity level of about 23 to 24 °Brix seeds are dark brown, corresponding to colour levels of 10, 11 and 12, therefore we have chosen to use 11 as the reference point for dark brown).

Example: Step 1: 20 seeds.
**Step 4 and 5:** (top surface) 5 seeds at colour 9, 11 at colour 10 and 4 at colour 11, the weighed mean for seed colour of the top surface is \( \frac{(5 \times 9) + (11 \times 10) + (4 \times 11)}{20} = \frac{199}{20} = 10 \)
(bottom surface) 5 seeds at colour 8, 12 at colour 9 and 3 at colour 10, the weighed mean for seed colour of the bottom surface is \( \frac{(5 \times 8) + (12 \times 9) + (3 \times 10)}{20} = \frac{178}{20} = 8.9 \)
**Step 7:** Overall seed colour is \( \frac{10 + 8.9}{2} = \frac{18.9}{2} = 9.5 \)
**Step 8:** Percentage of dark brown colouration is \( \frac{9.5}{11} = 86\% \)

**Method B**
1. Turn all the seeds so that the top surface is up.
2. For the group of seeds as a whole estimate the percentage of the total seed surface that is coloured dark brown that is, colours 10, 11 or 12 (see note in step 8).
3. Turn all the seeds so that the bottom surface is up and repeat step 2.
4. Average the two assessments to give the overall percentage of seed colouration.
References


