Alternatives to bunch thinning in yield control and its effects on quality of the grapes and wine composition in cv. Baga (*Vitis vinifera L.*)

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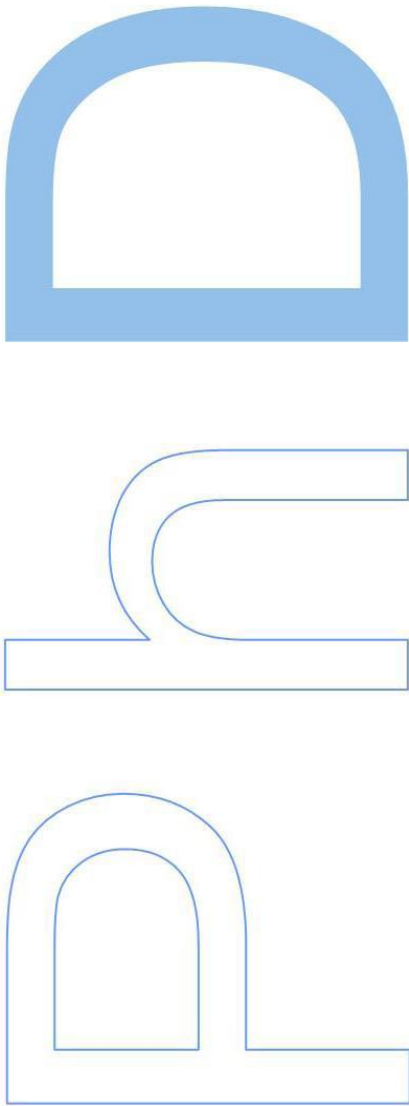

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Alternatives to bunch thinning in yield control and its effects on quality of the grapes and wine composition in cv. Baga (*Vitis vinifera* L.)

Métodos alternativos à monda de frutos no controlo do rendimento e gestão da qualidade da uva e do vinho, na casta Baga (*Vitis vinifera* L.)

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Publications

Silva HO, Baptista M, Guedes de Pinho P, Queiroz J. *Double Maturation Raisonnée*: effects on quality of grapes and wine composition in cv. Baga (Vitis vinifera L.). *Double Maturation Raisonnée*: Effets sur la qualité des raisins et la composition du vin de cv. Baga (*Vitis vinifera* L.). Proceedings 20th International Symposium GiESCO. Mendoza. 2017.

Silva HO, Baptista M, Moreira N, Guedes de Pinho P, Queiroz J. Three-year study of yield control methods and its effects on quality of the grapes and wine composition - cv. Baga (*Vitis Vinifera* L.). Proceedings of the 10th International Congress of Vitivinicultural Terroirs, Tokaj, 2014.

Silva HO, Baptista M, Guedes de Pinho P, Queiroz J. Alternatives to bunch thinning in yield control and its effects on quality of the grapes and wine composition in cv. Baga (*Vitis vinifera* L). Alternative à l'eclaircissage des grapes pour le controle du rendement et ses effets sur la qualité des raisins et du vin, cv. Baga (*Vitis vinifera* L). Proceedings 18th International Symposium GiESCO (Porto), Ciência Téc. Vitiv. 2013; 28: 436-440.

Queiroz J, Silva HO, Baptista M, Baptista M, Guedes de Pinho P. Alternatives to bunch thinning in yield control and its effects on quality of the grapes and wine composition in cv. Baga (*Vitis vinifera* L). Proceedings of the 9th International Congress of Vitivinicultural Terroirs, Dijon-Reims, 2012: 6. 49-52.

Abstract and keywords

As it happens with other grape varieties, Baga is prone to high productivity and also to rot, due to the production of compact clusters and thin skin berries. The occurrence of some diseases and excessive productivity are frequently associated, and therefore, yield control is an important issue. Fruit thinning and the application of growth regulators do not produce intended effects, revealing inconsistencies, technical difficulties to implement and high costs. Manual cluster thinning is time-consuming and chemical thinning is difficult to control, yielding random results depending on variety, phenological status and dosage of the used chemicals.

A three-year study was carried out, testing the production control techniques "early severe leaf removal" (manual and mechanical, MAD and MED, respectively), "*Double Maturation Raisonnée*" (DMR) and "manual bunch thinning" (MBT).

The objective of this work was to compare the effects of MAD, MED, DMR and MBT as methods of yield control and to demonstrate their individual effects on Baga variety, quality of grapes, musts and resulting wines. Using MAD, the first six basal leaves were removed at flowering, while MED was performed using the leaf removal machine in the fruiting zone on both sides of the vine at the same period. MBT was performed at *veraison*, limiting one bunch per shoot. DMR was performed 15 days before harvest, leaving shoots and bunches hanging on the wires.

A randomized complete block design with four replications was used.

The results reflected some influence of the different climatic conditions over the effects of each method studied on three harvests. However, some tendencies could be found: the techniques studied decrease vine yield; MAD, MED, DMR and MBT showed successfully lower yields; MAD, MED and DMR showed lower incidence of rot and cluster compactness, as well as some improvements in the composition of the must (something not presented in MBT); the wines produced with DMR and MED presented good sensorial quality, similar or superior to the used commercial References, while wines produced under MAD and MBT conditions presented superior results compared to Control (CTR) but not to the References; MED proved to be a lower-cost alternative to the other methods.

Keywords: Yield; Quality; Bunch Thinning; '*Double Maturation Raisonnée*'; Early Defoliation.

Resumo e Palavras-chave

Tal como ocorre com outras variedades de uva, a casta Baga é propensa a produtividade elevada e também à podridão, devido a produzir cachos compactos e bagos de película fina. A ocorrência de algumas doenças e produtividade excessiva estão frequentemente associadas, sendo, por isso, o controle de rendimento uma questão importante. A monda de cachos e aplicação de reguladores de crescimento não produzem os efeitos pretendidos, revelando inconsistências, dificuldades técnicas de implementação e custos elevados. A monda manual é demorada e a monda química é difícil de controlar, produzindo resultados aleatórios dependendo da variedade, estado fenológico e de dosagem dos químicos usados.

Foi realizado um estudo de três anos, testando as técnicas de controlo de produção "desfolha precoce severa" (manual e mecânica, MAD e MED), "*Double Maturation Raisonnée*" (DMR) e a “monda de cachos manual” (MBT).

O objetivo deste trabalho foi comparar os efeitos destas técnicas MAD, MED, DMR e MBT como métodos de controle de rendimento e demonstrar os seus efeitos sobre qualidade das uvas, mostos e vinhos da casta Baga. Utilizando MAD, as primeiras seis folhas basais foram removidas à floração, enquanto que MED foi realizada utilizando a máquina de desfolha na zona de frutificação em ambos os lados da videira no mesmo período. MBT foi realizada na fase do “pintor”, limitando um cacho por pâmpano. A técnica DMR foi realizada 15 dias antes da colheita, deixando varas e cachos pendurados nos arames. Foi utilizado o delineamento experimental de blocos ao acaso (aleatório), com quatro repetições.

Os resultados obtidos, sobre os efeitos de cada um dos métodos em estudo, foram influenciados, em certa medida, pelas diferentes condições climáticas das 3 colheitas. No entanto, algumas tendências podem ser encontradas: as técnicas estudadas fizeram diminuir a produtividade da videira; tendo a MAD, MED, DMR e MBT mostrado menores rendimentos; a utilização das técnicas MAD, MED e DMR resultou numa menor incidência de podridão e compacidade do cacho, e em algumas melhorias na composição do mosto (algo não apresentado por MBT); os vinhos produzidos com uvas sujeitas às técnicas de DMR e MED apresentaram boa qualidade sensorial, similar ou superior às Referências comerciaisutilizadas, enquanto as obtidas pelas técnicas de MAD e MBT apresentaram resultados superiores ao CTR mas não às Referências; a técnica de MED revelou ser uma alternativa de menor custo aos outros métodos.

Palavras-chave: Produção; Qualidade; Monda de cachos; ‘*Double Maturation Raisonnée*’; Desfolha precoce.

Index

Table of Contents

Table list

Figure list

Abbreviations

3MH - 3-mercaptohexanol

3MHA - 3-mercaptohexylacetate

4-EG - 4-ethylguaiacol

4-EP - 4-ethylphenol

4MMP - 4-mercapto-4-methylpentan-2-one

4-MMPOH - 4-mercapto-4-methylpentan-2-ol

ABA - Abscisic acid

BIO - Bio stimulant modality, *Ascophyllum Nodosum*

CBW - Canopy bottom width

CEPA - 2-Chloroethylphoshonic acid

CH - Canopy height

CSA - Canopy surface area

CTP - Canopy top width

CTR - Control modality

DMR - '*Double Maturation Raisonnée*' modality

DMR30 - '*Double Maturation Raisonnée*' of 30 days modality

DOC - Denominação de origem controlada

DOP - Denominação de origem protegida

E-L stage - Eichhorn Lorentz vegetative growth stages

ELS - Exposed leaf surface

ET_O - Reference evapotranspiration

EU - European Union

FVT - Free volatile terpenes

GA - Gibberellic acid

GC-MS - Gas chromatography-mass spectrometry

GDC - Geneva double curtain

GDD - Growing-degree days

ha - Hectare

HCl index - Hydrochloric acid index

hL - Hectolitres

HS-SPME - Head-space Solid phase microexctraction

IBMP - 2-methoxy-3-isobutylpyrazine

IPMP - 2-methoxy-3-isopropylpyrazine

LA - Leaf area

LA/Yield - Leaf area yield ratio

LLN - Leaf layer number

LN - Leaf number

MAD - Manual Early Leaf Removal modality

MBT - Manual Bunch Thinning/ Manual Cluster Thinning modality

MED - Mechanical Early Leaf Removal modality

MhL - Millions of hectolitres

MPa - Mega Pascal

NAA - Alpha-naphtaleneacetic acid

OAV - Odour activity value

PAR - Photosynthetically active radiation.

PC - Principal component

PCA - Principal component analysis

PQA - Point Quadrat Analysis

PTI - Potential Fertility Index

PVT - Potentially-volatile terpenes

r - Linear regression coefficient

RDI - Regulated deficit irrigation

SBMP - 2-methoxy-3-sec-butylpyrazine (3-sec-isobutyl 2-methoxypyrazine)

SPME - Solid-phase microextraction

T - Temperature

TA - Titratable Acidity

TDN - 1,6-trimethyl-1,2-dihydronaphthalene

TPB - (E)-1-(2,3,6-trimethylphenyl)buta-1,3-diene

TPI - Total Polyphenol index

UV - Ultraviolet radiation

VAZ - Violaxanthin, antheraxanthin, zeaxanthin pool

VSP - Vertical shoot positioning

%IC - Percentage of internal clusters

%IL - Percentage of interior leaves

%Gaps - Percentage of gaps of the canopy

Preamble

During the implementation of the work, the evaluation of results and the writing itself, I was continually rushed with doubt about the focus of this dissertation, the guiding principle, the juice that will nourish the knowledge of Baga, Bairrada region and Viticulture.

My closest family's life has been linked to the wine throughout my life. Since young age, I kept hearing discomfortable ideas about Bairrada and Baga, like both were united in a "Siamese connection": harsh, unexplained, undrinkable, retrograde and somehow exciting. These images were being cemented, my "knowledge" kept being nurtured based in third-party thoughts but always unfounded and inexperienced, without having the opportunity to be confronted with the question and being clarified.

During a complete decade, I discovered wine, wine styles, wine regions, grape varieties, vineyards, wineries, techniques and methodologies… and Baga remained unclear and undiscovered. After having accomplished some stages of academic training, with the wine always in the background (a journey made on the contrary, starting discovering the final product and walking backwards on the production chain), I finally decided that I should learn more about the 'origin of everything': grapevine and grapes. The Universe somehow pushed me to Bairrada, to the "damned" and misunderstood Baga!

It has been a pleasure to work and to meet a grape variety who most seem to reject, seeming like the 'outcast' of Portuguese grape varieties; getting to know a being with closed nature, bad-tempered, capricious, but that is also generous for those who devote time, attention and patience. Fortunately, my relationship with the 'friend' Baga did not ended after this work - I am fortunate to work professionally with Baga, in Bairrada, in vineyards over 60 years old, with other trellising, with other motivations, but always generous.

Finally, I tried to focus not only on numbers to support science but also in individual perception of wine; after all, grape quality and wine quality are not easily or fully explained by numbers and figures or translated into scientific writing.

Hugo Silva

I Overview

Grape and wine productions play a central role in Western society, today and also in the past. They have an important influence in several aspects of the society, such as economic, social, religious and intellectual.

Both grape and wine productions are economically important, particularly in the Mediterranean basin, and are also money-rewarding agricultural activities (when comparing with other agricultural productions). Beside these aspects, they are not accessible to everyone due to legal restrictions of vine plantation, of wine production and of wine commercialization, and also because they demand extensive knowledge and dedicated care.

Socially, wine is a beverage associated with key events like celebrations and agreement/pact/document signings, traditionally is used as distinguished offer, socially assumed to be associated with gentlemen behaviour, and some vine/wine expressions are popularly used with noble meanings.

Wine plays an important role in religion, frequently entitled 'the nectar of the Gods' and also as 'The blood of Christ' for the Christians.

Intellectually, wine is associated with intellectual beings, with reflection about life, with humanity and respect among men.

The great impact of wine in the History of Mankind explains the fact that we can find vines planted in almost every country of the planet.

According to '*Organisation Internationale de la Vigne et du Vin*' (OIV) data from 2017 [1], 267 million hectolitres (MhL) of wine were produced worldwide in 2016 (Table 1), with more than 80% of this volume being produced by only ten countries. Wine consumption worldwide has maintained near to 240 MhL per year, a constant trend since 2009 (Figure 1). Combining both data, it is clear that the problem of wine surplus is a current and global issue and it also highlights the need for an increased and urgent adaptation of the wine industry to the demand preferences. Data also shows that this reduction of global consumption is fairly related to the effects of the 2008 world economic crisis [1]. Finally, another feature of the data is the declining of wine production, and also consumption, in traditional wine producing countries, while it is increasing in the northern European countries and others, such as China and Australia (China is expanding the vine plantings surface, the volumes of wine production and the national consumption of wine are concomitantly increasing).

Table 1 - Wine production for 2012-16 (Source: Adapted from OIV, 2017 [1]).

Million hL	2012	2013	2014	2015	2016	2016/15 Var. Volume	2016/2015 Var %
Italy	45.6	54.0	44.2	50.0	50.9	0.9	2
France	41.5	42.1	46.5	47.0	43.5	-3.5	-7
Spain	31.1	45.3	39.5	37.7	39.3	1.7	4
USA	21.7	24.4	23.1	21.7	23.9	2.2	10
Australia	12.3	12.3	11.9	11.9	13.0	1.1	9
China	13.5	11.8	11.6	11.5	11.4	-0.1	-1
South Africa	10.6	11.0	11.5	11.2	10.5	-0.7	-6
Chile	12.6	12.8	10.0	12.9	10.1	-2.7	-21
Argentina	11.8	15.0	15.2	13.4	9.4	-3.9	-29
Germany	9.0	8.4	9.2	8.9	9.0	0.1	1
Portugal	6.3	6.2	6.2	7.0	6.0	-1.0	-15
Russia	6.2	5.3	4.9	5.6	5.6	0.0	0
Romania	3.3	5.1	3.7	3.5	3.3	-0.3	-8
New Zealand	1.9	2.5	3.2	2.3	3.1	0.8	34
Greece	3.1	3.3	2.8	2.5	2.6	0.0	2
Serbia	2.2	2.3	2.3	2.3	2.3	0.0	0
Austria	2.1	2.4	2.0	2.3	2.0	-0.3	-14
World Total (OIV)	258	290	270	276	267	-9	-3

Apart from the wine consumed, a substantial percentage of the wine produced is used as a by-product for other derivatives' wine associated products such as vinegars, distilled beverages, culinary sauces, etc. These data are usually missing in most of the global annual statistics so it is difficult to have a clear and overall vision about the wine production/consumption ratios. Objectively, the hypothesis that these sources of wine consumption would absorb the surplus of wine production in some years cannot be disregarded - this would explain why the global wine production is not dropping sharply as one would predict after successive years of wine surplus scenario.

Nevertheless, this would not happen in all years.

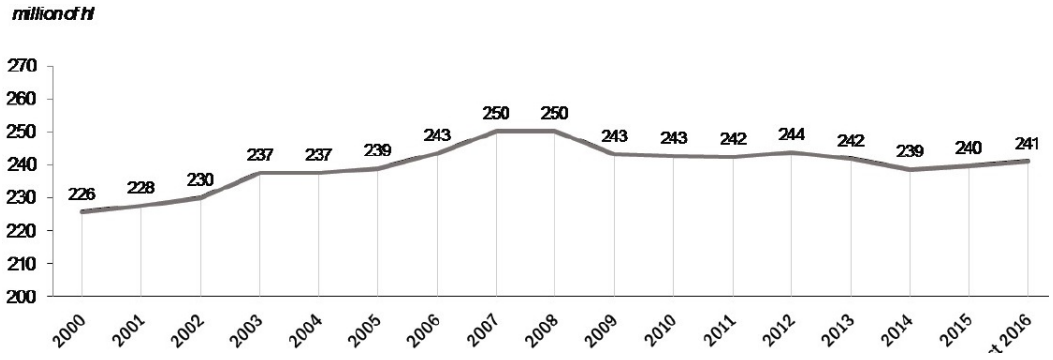

Figure 1 - World wine consumption, expressed in millions of hectolitres (Source: OIV, 2017 [1]).

Concerning the cultivated area with vines (Figure 2), it was over 7.5 million hectares (Mha) worldwide. The area had been increasing since 2011, the year of the lowest

value between 2000 and the present; Europe had the biggest relative reduction, almost 10 percentual points but still represents an absolute decrease of 17% in cultivated area - the OIV report [1] noticed that this decrease was the consequence of the abandonment of vineyards. In contrast, Asia has significantly increased its vineyard area. Again, it is difficult to explain the global increase in vineyard area (excluding Europe) when there is a global surplus in wine production.

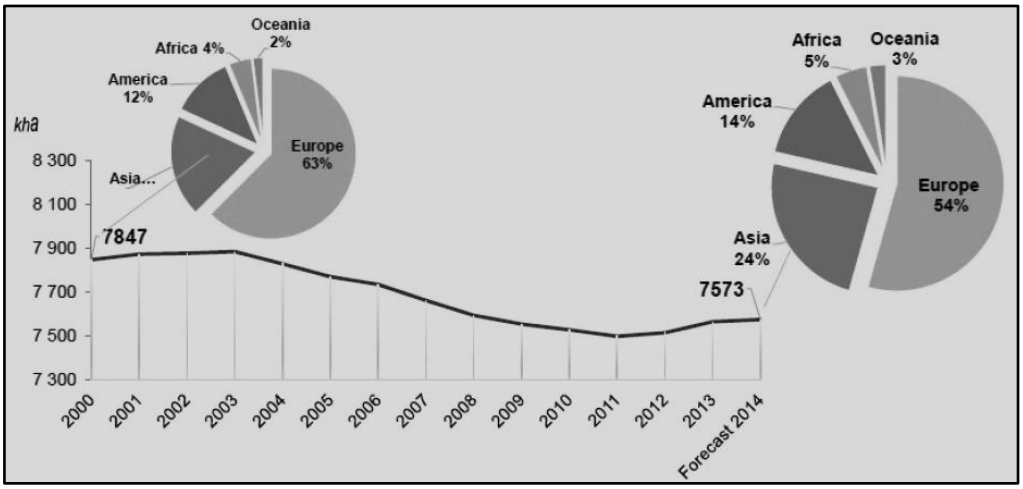

Figure 2 - Evolution of planted vines area (Source: OIV, 2017 [1]).

Another important feature that should be pointed out is that a substantial proportion of the wine produced globally is from a very limited number of varieties - 10 varieties represent close to 26% of the global planted area [2] (Table 2). The use of these more common and vastly planted grape varieties can bring, in the first years, some commercial impact for a new producer - it will attract the attention of variety-driven wine consumers; but, just after a few years in the business and if nothing other than the grape variety is added to the production (like branding, new products, new varieties, new wine styles, marketing and advertising), the attention brought by the varietal wines tend to abruptly disappear, because the consumer choice was only motivated by a feature that is not exclusive to that particular wine producer - the grape variety. The massive global offering of wine from these ten varieties, combined with a wine surplus scenario, can result in increased difficulties for all sorts of wine producing entities, regardless the country of origin or their size: cooperatives, very large producers, large and medium producers, small producers and boutique wineries.

In order to avoid economical unbalances, single countries and multi-country trade organizations are constantly establishing new regulations for the wine industry, acting specifically in limiting production and tightening quality standards - yield control and

quality assurance are important tools in the legally restricted nonetheless vast wine business world.

Table 2 - Most common grape varieties planted (in hectares, Source: Adapted from IVV, 2015 [2]).

Order	Grape Variety	Colour	2010 (ha)	2000 (ha)	1990 (ha)
1	Cabernet Sauvignon	Red	290 091	220 890	127 678
2	Merlot	Red	267 169	211 967	154 752
3	Airen	White	252 364	387 978	476 396
4	Tempranillo	Red	232 561	32 985	47 429
5	Chardonnay	White	198 793	145 344	69 282
6	Syrah	Red	185 568	101 516	35 086
7	Garnacha Tinta	Red	184 735	213 987	282 997
8	Sauvignon Blanc	White	110 138	64 889	44 677
9	Trebbiano Toscano	White	109 772	136 572	207 442
10	Pinot Noir	Red	86 662	60 099	41 539

Portugal's wine context is similar to the rest of the Europe: according to 2014 data from "Instituto da Vinha e do Vinho" (IVV) [2], there were 218 677 ha of vines planted, from each there were 79 573 ha of vines/vineyards with "Denominação de Origem Protegida" (DOP) certification (Table 3), that means "Protected Designation of Origin" or "Protected Geographic Indication".

Table 3 - Planted vine areas per Portuguese region (Source: Adapted from IVV, 2015 [2]).

Region	Area (ha)	
	DOP	Total
Minho	15 810	27 432
Tras-os-Montes	417	23 303
Douro	40 378	43 611
Beiras	8 370	52 670
Lisboa	1 074	22 425
Tejo	1 161	15 653
Península de Setúbal	2 154	8 622
Alentejo	10 090	23 188
Algarve	119	1 773
Total	79 573	218 677

There are 26 grape varieties with more than 1000 ha of vines planted each in Portugal, with Aragonez/Tinta Roriz/Tempranillo and Touriga Franca with more than 10 000 ha planted each (Table 4). Although Portugal has a large number of autochthonous varieties and several distinct DOP regions, there is a limited offer of wine producing varieties: the 10 most planted varieties in Portugal combined represent 36% of all vine area planted: Aragonez/Tinta Roriz/Tempranillo, Touriga Franca, Castelão/João de Santarém/Periquita, Fernão Pires/Maria Gomes, Touriga Nacional,

Trincadeira/Tinta Amarela/Trincadeira Preta, **Baga**, Síria/Roupeiro/Códega, Arinto/Pedernã e Syrah/Shiraz.

Table 4 - Most planted varieties in Portugal in 2014 (Source: IVV, 2015 [2]).

Grape Variety	Area (ha)	%
Aragonez / Tinta Roriz / Tempranillo	15292	7
Touriga Franca	12231	6
Castelão / João de Santarém / Periquita	9287	4
Fernão Pires / Maria Gomes	9126	4
Touriga Nacional	8183	4
Trincadeira / Tinta Amarela / Trincadeira Preta	7632	3
Baga	4996	2
Síria / Roupeiro / Códega	4909	2
Arinto / Pedernã	4244	2
Syrah / Shiraz	3925	2
Loureiro	3820	2
Alicante Bouschet	3710	2
Tinta Barroca	3646	2
Vinhão / Sousão	2953	1
Alvarinho	2224	1
Malvasia Fina / Boal	2094	1
Rufete / Tinta Pinheira	2078	1
Marufo / Mourisco Roxo	2064	1
Malvasia Rei	1897	1
Jaen / Mencia	1826	1
Caladoc	1781	1
Cabernet Sauvignon	1712	1
Rabigato	1553	1
Antão Vaz	1339	1
Trajadura / Treixadura	1171	1
Azal	1 080	0.5

Baga represents close to 5000 ha of the planted vineyard area in July 2014, and it is almost limited to the Bairrada and Dão regions. According to IVV reports, Baga planted area in July 2013 was 4811 ha [3], in July 2014 was 4996 ha, and 7105 ha in July 2015 [4] - these features are for a total vineyard area in Portugal, and not only Bairrada region. If one believes that the vast majority of Baga plantings occur in Bairrada region, this evolution represents an inversion of the apparent decrease of Baga planted area over the last decade in Bairrada (during the 80's, Baga represented 80 to 90% of the red varieties planted in Bairrada region [5]).

Portugal's overall production of wine is a bit more than 6 000 000 hectolitres (hL) per year (Table 5), divided between 2 227 500 hL of wine with DOP, 725 000 hL of fortified wine, 1 685 000 hL of wine with "Indicação Geográfica Protegida" (IGP) and the rest just wine (close to 1 500 000 hL). Minho, Douro and Alentejo regions are responsible

for the major quota of the DOP wine produced, and the central-southern region produces the most important amount of IGP wine produced (Alentejo, Lisboa, Península de Setúbal and Tejo regions). Vinho do Porto is the most important fortified wine with DOP produced in Portugal, accounting for more than 700 000 hL. Interesting information is that Portugal produces less wine without DOP or IGP than wine with DOP and wine with IGP.

Table 5 - Wine production in Portugal in 2014 per region (Source: IVV, 2015 [2]).

Region	Total	DOP wine	DOP fortified wine	IGP wine	Wine with indication of Year/Variety	Wine
Minho	793 417	743 626	0	43 837	0	5 953
Trás-os-Montes	96 615	12 070	0	7 600	1 204	75 741
Douro & Porto	1 516 925	507 497	707 752	42 631	0	259 045
Beira Atlântico	255 333	71 787	0	28 633	34 263	120 649
Terras do Dão	304 824	195 362	0	30 702	4 887	73 873
Terras da Beira	215 783	42 853	0	35 930	0	137 000
Terras de Cister	64 731	21 266	0	1 400	0	42 064
Tejo	500 807	54 860	276	159 846	3 030	282 795
Lisboa	885 742	46 911	477	503 749	757	333 848
Península de Setúbal	407 853	108 522	15 624	190 009	30	93 668
Alentejo	1 127 910	469 652	433	633 255	150	24 419
Algarve	11 676	1 668	0	7 794	0	2 214
Continental Subtotal	**6 181 615**	**2 276 075**	**724 562**	**1 685 388**	**44 321**	**1 451 271**
Madeira	43 136	1 270	39 426	35	0	2 405
Açores	6 595	53	1 219	973	0	4 350
Island Subtotal	**499 731**	**1 323**	**40 645**	**1 008**	**0**	**6 755**
Total	**6 231 347**	**2 277 398**	**765 207**	**1 686 396**	**44 321**	**1 458 026**

Portugal's wine production is slowly decreasing (Table 6), the same occurring with Bairrada/Beira Atlântico region. The Bairrada/Beira Atlântico region is responsible for producing 255 333 hL in 2014 vintage, from which 71 787 hL was produced as DOP wine, 28 633 hL as IGP, and the rest as table wine.

Table 6 - Evolution of wine production in Portugal per region 2004-14 (Source: IVV, 2015 [2]).

Region	2004/05	2005/06	2006/07	2007/08	2008/09	2009/10	2010/11	2011/12	2012/13	2013/14
Minho	987 715	939 564	937 605	710 625	784 028	866 985	912 176	823 341	655 253	793 417
Trás-os-Montes	225 787	255 798	232 042	98 302	105 075	110 614	119 367	102 005	108 615	96 615
Douro & Porto	1 645 627	1 743 865	1 717 728	1 443 429	1 379 051	1 351 949	1 660 408	1 329 423	1 346 152	1 516 925
Beira Atlântico	377 947	413 322	365 030	255 978	211 669	246 705	297 704	292 596	283 897	255 333
Terras do Dão	376 121	487 491	515 551	240 723	251 863	297 483	355 687	293 537	356 454	304 824
Terras da Beira	364 606	356 079	363 100	125 789	194 365	192 084	224 735	184 759	217 693	215 783
Terras de Cister	77 650	97 046	94 312	37 605	78 831	47 872	61 036	45 959	64 655	64 731
Tejo	845 425	685 319	639 747	669 472	518 989	544 935	630 548	382 276	641 789	500 807
Lisboa	1 294 856	1 177 088	1 195 983	1 056 407	932 736	962 323	1 204 098	826 666	1 097 712	885 742
Península de Setúbal	373 125	338 204	428 488	418 989	337 139	379 371	431 696	308 857	517 797	407 853
Alentejo	825 709	693 364	961 721	930 452	811 690	810 338	1 189 719	969 832	970 124	1 127 910
Algarve	24 107	27 955	31 672	27 587	23 698	23 650	19 190	13 150	12 338	11 676
Continental Subtotal	**7 418 676**	**7 215 095**	**7 482 979**	**6 015 360**	**5 629 135**	**5 834 310**	**7 106 363**	**5 572 402**	**6 272 479**	**6 181 615**
Madeira	41 213	42 656	49 245	45 591	49 925	45 449	36 782	38 769	49 637	43 136
Açores	21 339	8 493	10 482	12 091	9 500	13 754	4 783	11 192	4 991	6 595
Island Subtotal	**62 552**	**51 149**	**59 728**	**57 682**	**59 426**	**59 203**	**41 564**	**49 961**	**54 628**	**49 731**
Total	**7 481 228**	**7 266 244**	**7 542 706**	**6 073 042**	**5 688 560**	**5 893 513**	**7 148 927**	**5 622 363**	**6 327 107**	**6 231 347**

Comparing Portuguese wine production with other European Union (EU) countries (Table 7) and other countries from the world (Table 8), Portugal produces significantly less wine than Germany, France, Spain and Italy, and also South Africa, China,

Australia, Chile, Argentina, and United States of America - Portugal's wine production represents 2% if combining the productions of these countries.

Table 7 - Evolution of wine production in European Union (Source: IVV, 2015 [2]).

	2009/10 (1000hL)	2013/14 (1000hL)	5-Year variation (%)
Others	1 259	1 143	-9
Slovenia	754	770	2
Croatia	1 424	1 248	-12
Bulgaria	1 246	1 755	41
Austria	2 352	2 392	2
Hungary	3 198	2 666	-17
Greece	3 079	3 343	9
Romenia	6 703	5 242	-22
Portugal	5 894	6 231	6
Germany	9 228	8 409	-9
France	46 743	41 491	-11
Spain	39 232	52 460	34
Italy	50 665	54 029	7
UE Total	**171 777**	**181 179**	**5**

In this scenario, combining wine surplus with the small size of the country and its wine production, Portuguese wine companies face the difficult challenge of creating a noticeable place in the global market of wine.

Table 8 - Comparison of Portuguese wine production with other countries (Source: IVV, 2015 [2]).

	2009/10 (1000hL)	2013/14 (1000hL)	5-Year variation (%)
Others	41 692	38 580	-7
Portugal	5 894	6 231	6
Germany	9 228	8 409	-9
South Africa	9 986	10 980	10
China	12 800	11 780	-8
Australia	11 784	12 310	4
Chile	10 093	12 846	27
Argentina	12 135	14 984	23
USA	21 965	23 500	7
France	46 743	41 491	-11
Spain	39 232	52 460	34
Italy	50 665	54 029	7
World Total	**272 217**	**287 600**	**6**

One alternative to increase Portugal's importance in the wine world is to promote the wine as a wine country, rather than promote individual companies - IVV, Viniportugal and DOP boards had only started to walk this path.

One other path that could help to achieve wine companies' success is increasing quality and gradually increasing selling price of the Portuguese wines. This purpose is usually accompanied of lowering yields per area of vineyard and increasing viticulture/winemaking knowledge and technical capacity.

Bairrada region is one of the oldest wine producing regions of Portugal: wine production in this region goes back to romans time. Several Portuguese Kings took measures to protect the wines from the area which is presently the Bairrada region due to their quality and social-economic importance, even though the vineyard destruction imposed by Marquês de Pombal following the establishment of the Vinho do Porto region. The area of the region spreads from 'Minho' to 'Estremadura', and it can be described by its small properties of intensive and multicultural agriculture. The DOP Bairrada region is situated between Águeda and Coimbra, delimitated at North by the Vouga river, at South by the Mondego river, at East by the Caramulo and Buçaco mountains and at west by the Atlantic Ocean. The region is constituted mainly by plains, with vines planted usually below 120 meters' altitude; due to the plain and the closeness to the ocean, the climate is temperate with strong maritime influence, with abundant rains and cool temperatures; the winters are long and cool, and the hot days of the summer are smoothen by cool sea breezes. There are several soils profiles that go from the argyle-calcareous to sandy soils, giving different styles of wines - normally fresh, acidic and low alcohol, with fruity and mineral whites, vibrant sparkling wines and highly tannic reds.

The medium to small size properties establish grounds for the presence of large cooperatives and large wine companies - the highly fractioned grape production of the region is bought by the large wine producers, so the main objective of grape producers is to produce more grapes to sell.

In this context, Baga variety rose between all other varieties planted in the region - because is a highly productive variety, it was called in the Bairrada region as '*Paga-dívidas*' (Pays-debts) and '*Carrega-burros'* (Burdens-donkeys)[6]. Baga was then the most important red variety of the Bairrada region. Nowadays, the importance of the variety diminished due to several aspects: the quality of wines produced from Baga cannot be reliably predicted before the harvest, because high yield production is usual, maturation of the fruits is every year a challenge (sometimes incomplete), significant fraction of grapes has clusters of *Botrytis* infection if rain occurs after *veraison*, which require tremendous work with canopy management because shoots grow unevenly and horizontally (shoot trimming, leaf removal and fruit/bunch/cluster thinning are frequently needed), and, of course, because of planting of new vineyards. In order to counteract these problems and be successful, substantial investment in labour has to be done by the grape producer, but the quality of the wines continues to be unpredictable until the day of the harvest.

Due to these profit-debilitating problems, Baga vineyards are being to be planted with other grape varieties. This is particularly observed for those vineyards of old age plants, resulting in a region that loses its identity, strongly related with Baga variety.

In a perspective of excessive wine production and world wine surplus, it is important to know its impact according to some aspects: relation to legal problems, production and also with commercial value of the product:

a) Legal production limits imposed by international and national regulators, and also by regional regulators of a particular region/DOP, aim to regulate the total yield per vine area, in order to guaranty an appropriate quality level and also to prevent the fall of commercial price of wines from a specific region. Bairrada region DOP wine is regulated by Portuguese law, and the yield for red wines is limited to 80 hL per ha [7];

b) Yield limits also help to normalize standards' levels of production costs because avoid surplus in grape production benefits the regulation of the selling prices of grapes, aiming to create a competitive edge for the grape producers.

c) Yield limits determine a maximum volume of wine to be produced each year in a particular region. Because of the limited volume of wine available, the ratio between supply and demand is sustained (or even the demand increases, in the optimal situation) and, if quality standards are high or increasing gradually, the value of the product also increases - this helps to maintain/increase commercial prices for the wines of the region. These limits also help the producers to manage their production because production (and prices) fluctuations are usually due to climate variations.

Consequently, grape yield are controlled, and if needed, reduced. This is essential for small wine productions (regions and/or small countries).

For a grape producer, what kind of yield control or yield reduction strategies can be implemented?

Yield control strategies can be approximately divided into 3 major groups: long term methods (that relate to vineyard selection and planting); usage of some kind of product/mixture/etc; and soil/canopy management techniques.

These yield control strategies will be explained in further detail in the following chapters. At this point, it is important to emphasize that, adding to the goal of controlling the yield, each of these strategies have other consequences that have to be considered when making the decision - the consequences can be related to viticulture management, regarding the labor allocation and costs, of the plant response during the current vintage and for the subsequent vintages; which can consequently affect grape and wine quality.

Most of viticultural aspects that are influenced by using one of the mentioned strategies/techniques can be measured and sometimes predicted, even if takes several years to perform the measurement; on contrary, the aspects involving fruit and wine quality are difficult to be measured and/or predicted - as there are too many factors that have a determinant influence in wine quality (factors regarding viticulture, biochemistry, chemistry, sensorial, cultural and hedonic perception) some other factors related to wine quality also, are too subjective to be described and measured in an accurate way. Viticulture should be complementary to winemaking and may determine wine producing goals, through being able to pursue the strategies needed to obtain fruits that are able to produce the intended wine; but then, viticulture should also be useful to typify the virtues of the grapes produced in a particular vintage in order to predict the quantity and the qualities of the wines that can be produced with those fruits. Extensive and more complex relationship between viticulture and winemaking should be pursued in order to aid the decisions of the winemakers but also to empower their decisions, that is, to provide enough information to the winemakers in order that render them able to consciously make decisions leading to produce the wines they want.

In view of this local and economic context, the need arose for a study with the following objectives:

Compare the effects of the techniques Manual Early Leaf Removal (MAD), Mechanical Early Leaf Removal (MED), Manual Bunch Thinning/ Manual Cluster Thinning (MBT), and '*Double Maturation Raisonnée*' (DMR) as methods of yield control and to demonstrate their effects on the quality of grapes, musts and wines of Baga variety;

To use new techniques of yield control by means of expeditious means ('*Double Maturation Raisonnée*' - DMR) or mechanizable (Mechanical Early Leaf Removal - MED);

To test and to prove the effects of the Early Severe Leaf Removal and the '*Double Maturation Raisonnée'* as alternative methods of control of the production, comparing with Manual Bunch Thinning;

To demonstrate the respective effects on quality of musts and wines, produced with Baga variety, produced in the demarcated region of Bairrada;

Chemical and aromatic characterization of wines produced with Baga variety, and to elucidate some aspects related to the aromatic typicality of the Baga variety.

II Yield control techniques and wine quality

2.1 Reproductive cycle of the vine

Vineyard and vine management have always immediate consequences, some predictable, some unclear, and almost all management techniques used currently can carry consequences for several years after being employed. The knowledge of the grapevine yearly cycle can be useful to understand and to predict some of the consequences of vineyard and canopy management techniques.

The grapevine is a perennial plant, specially adapted to temperate climate - it has a vegetative growth active period (from spring to fall), and a resting period, during the winter. After bud burst in the spring, a complex bud is formed in young shoots, in the axil part of each leaf. In the bud is formed three latent buds - primary, secondary and tertiary buds. This bud may also burst within the same growing season, developing as a lateral shoot [8]. During the beginning of the growth period, several leaves primordia develop in each of these buds, while inflorescence primordia develop mainly in the primary bud [9]. By mid-summer, the latent buds enter in a paradormancy stage, in which bud burst is inhibited by factors originated in other plant organs, such as auxins from the apical meristem [10]. Entering fall, the decrease of sunshine hours and ambient temperature induce bud endodormancy, a phase in which bud burst is repressed by factors within the bud [10,11] - it will be required exposure to cold temperatures during a significant period to occur the bud evolution through the dormancy cycle and eventually be released from endodormancy [10,12]. Once the low temperature necessities are fulfilled, buds become capable of burst under the adequate conditions - buds can go through ecodormancy stage, as long as the environmental conditions inhibit bud burst because of being inadequate for supporting the plant growth [11].

The reproductive cycle of *Vitis vinifera* is an intricate complex process, covering over two years, deeply influenced by environmental conditions and cultural practices. During the flower formation (a process dependent of numerous factors [13]), four phases can be defined as crucial: induction, initiation and early differentiation (occurring during the first year), and differentiation after bud break (occurring in the second year) [14] (Figure 3).

During the first year, induction occurs, which means the formation of the reproductive primordia. Stems and inflorescences have a common primordia origin and successful destine depends on hormonal balance cytokinins and gibberellins - cytokinins favour the transition to flower. After the induction of the primordia of

inflorescence, stems and shoot, meristems that will convert into flower in the second year begin to develop, although not all will be present in the latent bud.

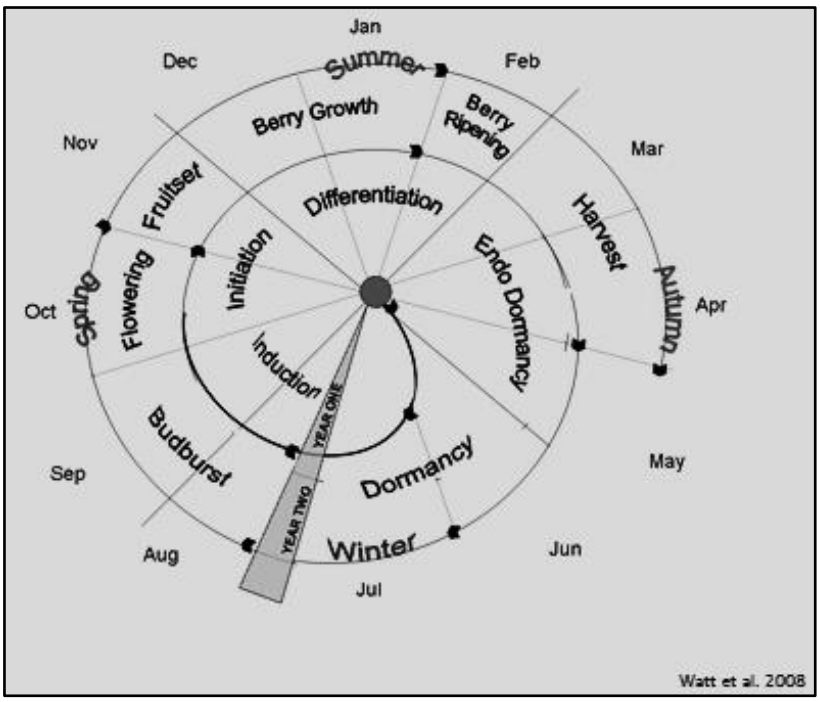

Figure 3 – Grapevine two-year lifecycle, according to Watt *et al* [14] - adapted for southern hemisphere.

The main factors regulating the fertility of the buds are light exposure [15,16,17], temperature (between 20 and 35º C) and carbohydrates availability. The absence of any kind of stress [18,19,20] and precipitation will also help to a successful initiation.

During growth, the dormant buds predominantly obtain carbohydrates from the leaves of the same side of the shoot [21]. There is a lack of competition between initiation and inflorescence differentiation in dormant buds [22,23], with the development of the flowers already open before the fruit set; on the other hand, there is evidence of competition between vegetative growth and flowering process [24]. Thus, a decrease in photosynthesis during flowering can produce negative impact on the fertility of the bud, although can also be related with the carbohydrates reserves in the perennial parts of the plant [25,26].

The formation and differentiation of the different parts of the flower begins after bud break in the second year. Light exposure, temperature and carbohydrates availability are key factors that influence bloom (same as for induction, initiation and differentiation of the first year), and carbohydrate availability is accepted to be the start trigger [15,16,17]. Low temperature and humidity combination can be negative for bloom, and can result in poor pollination and fruit set. It was also referred that rain periods before and during flowering can cause flowers to drop [27], before even opening. In theory, the

carbohydrates needed for flowering could come from the reserves of the perennial parts and from photosynthesis, although being widely accepted that the main source of carbohydrates for flowering is photosynthesis [28]. Some authors refer that leaf removal close to flowering period could cause abortion of flowers [25] and the decrease of fruit set. Other authors refer that an imbalance of macro and micronutrients can also produce negative impact in flowering and fruit set [29,30,31,32,33].

The transformation of the flower into a fruit, or fruit set, varies depending of the variety and clone. When flowering occurs under normal conditions, the decrease of fruit set is usually due to deficient nutrition of the flower and the newly formed fruit, not having enough energy to guaranty regular fruit development [34,35,36]. The occurrence of any irregular environmental condition or the usage of a cultural technique that lowers photosynthesis and/or sugar available in the plant will cause the decrease of fruit set.

After flower fecundation, fruit set and fruit development begins with a cell multiplication phase (increase in cell number by mitosis), which takes place during 2 to 3 weeks. Afterwards, cell multiplication ends and cell enlargement stage initiates, throughout approximately 4 weeks. At this point, cell number of pericarp is already definitive and so the berry size is dependent of the elasticity of the cell walls (and the cell number) [37]. If some irregular environmental condition or a cultural technique is performed that lowers photosynthesis and/or sugar available in the plant, a decrease of berry size will be caused.

2.2 Microclimate and canopy management

The importance of microclimate in viticulture was widely studied, like by Smart [38] among others, explored the effects of environmental conditions near and close to the clusters and leaves. Microclimate depends predominantly on leaf density and on the arrangement of leaves and clusters, which is critical to the quantity and quality of the light that reaches the plant, the leaves and the clusters, and also for the temperature and humidity surrounding them [39].

The sanitary condition of the grapes is a crucial element for their quality. For varieties with high leaf density, leaf transpiration can lead to the increase of humidity close to leaves and clusters and conditions prone to disease incidence; if the canopy is open and porous, ventilation is stimulated and more intense, consequently reducing humidity, decreasing the presence of fungal infections such as *Botrytis cinerea* [39,40,41].

Several authors [42,43,44,45,46,47] associate higher light exposure of the clusters with improving the quality of the grapes, referring higher content of sugar, polyphenols and anthocyanins, and lower malic acid concentration and titratable acidity.

In cooler climates, higher concentration of anthocyanins (related with colour development) is associated with increased cluster exposure to sunlight [48,49,50]; in warm regions, higher exposure can be extreme, causing berry skin sunburn [50,51,52] and lower colour [50,53,54]; sunlight exposure and concomitant increased temperatures were also described as key factors stimulating the degradation of malic acid [55,56] and it was also observed a greater assimilation of carbon dioxide in the synthesis of tartaric acid in berries exposed to sunlight, compared with berries under shading [57].

Grape phenolics are often mentioned as quality indicators specially for red varieties [58,59,60]. The influence of temperature and light on polyphenols synthesis and accumulation is a complex phenomenon because the metabolic pathways of these compounds are sensitive to both factors [52] and it is not simple to study these factors independently. In general, it is established that an increase in light exposure favours higher concentrations of berry polyphenols, specially flavonoids (anthocyanins and flavonols, mainly [52,53,54,61]). Moreover, negligible flavonol contents were found in berries that were not exposed to light [62].

From several studies designed to study the effects of light and temperature on anthocyanins accumulation [50,52,54,61], it was reported that the normal canopy conditions allow clusters to obtain sufficient light, with moderate intensity, not being a limiting factor; however temperature seems to be a limiting factor [47]. The temperature range of 17 to 26º C would be suited for the enzymes involved in the biosynthetic pathway [63]; 20 to 30º C for Cabernet Sauvignon [64] and for Merlot [52]. Temperatures around 35º C and high light exposure may lead to insufficient colour accumulation because of the inhibition of the anthocyanin synthesis and/or due to the increased degradation rate [54]. It was also found that the temperature range effect over the synthesis of anthocyanins depends on the variety [65].

Canopy might be defined as the leaf and shoot system of the vine [66]; it is described by dimensions of the boundaries in space (width, height, length, etc.) and also by shoot system (usually leaf area). Canopies can be continuous (the foliage from adjacent vines of the row combines, having no large gaps) or discontinuous (canopies are separated from vine to vine).

Canopies can be divided (one vine or adjacent vines are divided into discrete foliage walls) or dense (high leaf area within the volume bounded by canopy surfaces) - high

value of the ratio leaf area/canopy surface area (LA/CSA) [67], or of leaf layer number (LLN) or shoot density (shoots/m canopy) [68].

'Canopy management' includes a range of techniques which can be used in vines to alter the position or amount of leaves, shoots and fruits in space, to achieve some desired arrangement (i.e. canopy microclimate): winter and summer pruning, shoot positioning, leaf removal (principal and lateral leaf removal), shoot vigour control (suckering, shoot trimming, top trimming) and fruit thinning. Canopy management techniques can be used to improve production and/or wine quality, reduce disease incidence, facilitate labour and mechanisation. Open canopies also lead to more efficient distribution of agricultural chemicals [69].

Some basic principles of canopy management can be mentioned [38]:

- exposed leaf surface (ELS) should be healthy and efficient through all vegetative season;
- broad and well exposed ELS are desirable;
- keep cluster light microclimate suitable for the vineyard region;
- find balance between ELS and yield;
- canopy should help vineyard tasks and mechanization.

2.3 Wine quality and grape composition

What is wine quality?

Can basic, premium and ultra-premium wines be differentiated, chemically and by taste? Will they have the same quality standards or composition?

Do young and aged wines have the same qualities?

Does the quality standard has to be universal, or is it individual?

Are there some individual molecules that are responsible for highlighting an individual wine as high quality, or young wine, or as a cheap wine?

And, again, can we define '*quality*'?

The '*quality*' may be defined as "*A distinctive attribute or characteristic possessed by someone or something*" [70]; this definition is originated from Latin '*qualitas'*, meaning "*of what kind, of such a kind*". Clearly, '*quality*' means the intrinsic attributes of someone or something, without classifying if those attributes are positive or negative. There is another definition, more common, for '*quality*' - "*The standard of something as measured against other things of a similar kind; the degree of excellence of something*" [71]. Using this definition of quality, the subject is classifying the attributes as positive so 'quality wine' means that it is a very good wine when compared with other wines. The problem with the use of this terminology is that the attributes used to define if the wine has quality or not, are subjective, individual and not identified - each individual has a set of attributes to characterize a wine as good or not, and each individual experiences in a unique way a particular wine, so there are distinct and individual definitions for a single attribute due to physiological and neural abilities, cultural and experience learning and individual hedonic preferences.

Quality definition should be characterized by identifiable and measurable attributes, related to compound concentrations, consumer preferences, sensorial description/analysis or commercial value. For example, wine chemical composition can be a starting point to define wine quality, but it will not be enough to describe this quality. After clearly defining what does one mean as 'wine quality', the features that contribute positively or negatively to wine quality can be found, identified and related with the consumer perception of the wine. Perceived wine quality and their related compounds can be dependent of many aspects of the wine production, from grape composition on the moment of picking and varietal and vineyard characteristics, harvest conditions, fermentation conditions and winery techniques and maturation and conservation of the final wine.

But, complicating more, will the quality definition in grapes have the same parameters as for quality definition in wines? How can we estimate quality improvement of different viticultural technique or when we test a commercial product inside the winery?

Defining grape and wine quality is crucial to answer the previous questions, but they are very difficult to achieve. One first step could be to do a grape/wine composition assessment in order to identify positive and also negative attributes. Grape is the lead protagonist in viticulture and in wine production but is somewhat undervalued, when compared to wine - performing simple grape analysis when preparing the harvest and starting the wine production seems to be sufficient for wine producing companies: apparently, probable alcohol/sugar content, pH, and acidity are sufficient to ensure good planning and execution of the production of wine for many wine producers.

The technical conditions of the wineries have improved substantially in these last 3 decades, being difficult to find at point of sale wines without a minimum quality value, with gross defects or problems that resulted from wrong wine production - but little effort and investment was made for defining grape quality, and identifying grape attributes that are positive (or negative) for wine production.

2.3.1 Grape composition and berry development

The berry growth curve has four specific and distinct phases (Figure 4):

Phase 1, the berry formation phase, when the berries are still green;

Phase 2, the lag-to-*veraison* phase, when the bunch is completely formed, with berries fully grown and still green, until colour formation (*veraison*);

Phase 3, the ripening phase, when berries are coloured, acidity reduces, sugar and aroma contents increase, skin softens;

Phase 4, the over-ripening phase, when berries dehydrate, skin shrivels, sugar increases concentration, acidity lowers further [72].

Summarizing, the berry formation phase is characterized by cell division, followed by cell enlargement; the ripening phase is exclusively defined by cell enlargement, while the ‘over-ripening’ phase is described by berry water loss due to evaporation under hot and dry conditions (‘withering’). This later feature can be used for the production of late-harvest wines and ‘Passito’ wines, which is not be so interesting for table wines.

During the first phase, lasting 60 days after flowering approximately, the berry is formed, and the seed embryos created; the cell division is fast during the first few weeks and the total number of cells in the berry has been fully established [73]. The total

number of the berry' cells can be a limiting factor for the future size of the berry. After cell division, only cell enlargement occurs, even during the final stages of the berry formation phase. Between berry formation and ripening, *veraison* occurs - berry tissues become softer (skin and pulp) and change colour.

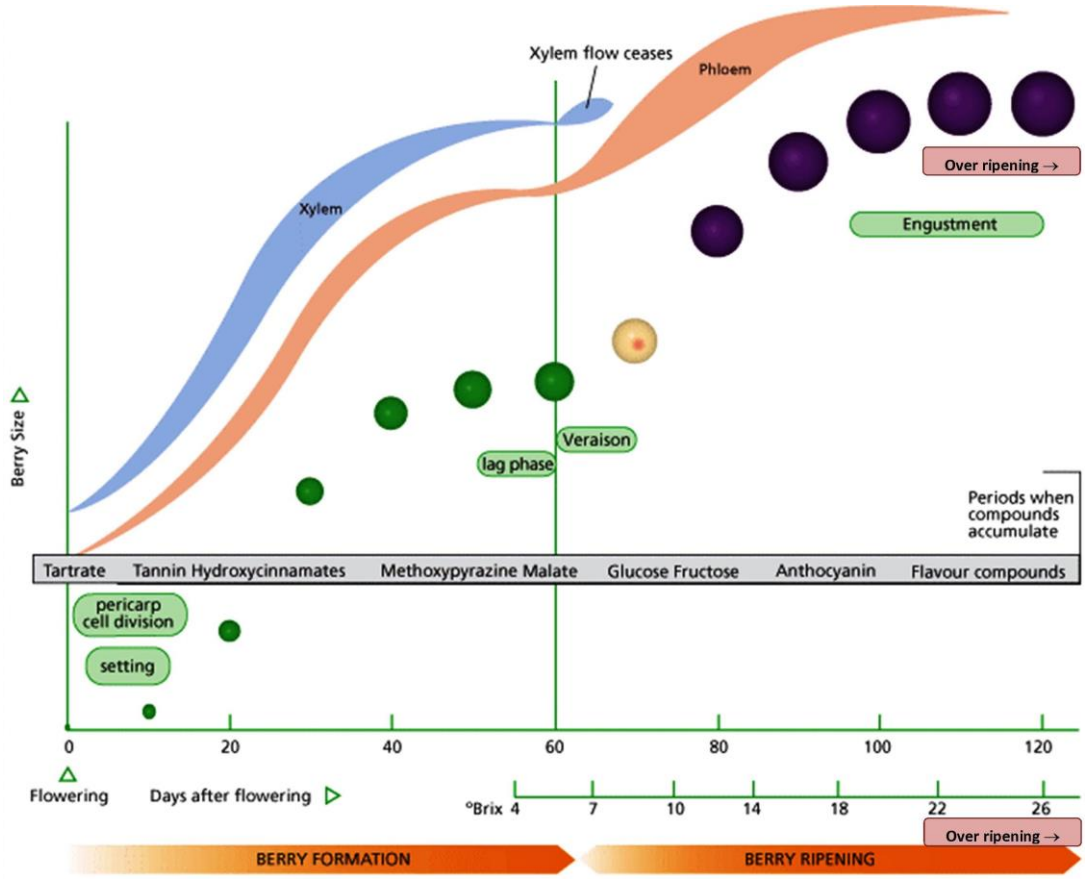

Figure 4 - Berry growth phases (adapted from James Kennedy) [74].

The nutritional needs of the grape berries are satisfied through the stem/pedicel, by a vascular system composed of xylem and phloem - xylem vessels transport water, mineral, growth regulators and nutrients from the root system to the rest of the plant; phloem vessels transport sugars (photosynthate) from the canopy. There is some evidence that xylem is functional in the grape berries until *veraison*, and largely reduced afterwards (to zero) [75]; phloem has little importance during the berry development phase but becomes the primary source of nutrients after *veraison*.

The enlargement of grape berries after *veraison* is primarily due to higher water content, associated with increased sugar content; there are some grape varieties (like Syrah) in which an increase of the sugar content at latter stages of ripening is not accompanied with the increase of berry volume, but by occurring berry shrinkage apparently due to loss of water by transpiration process [76,77,78]. This feature might

suggest that phloem activity can become limited or null in later stages of berry ripening, with berry dehydration becoming the most important mechanism until harvest (maybe achieving full maturation or continuing to over ripening).

Research works, studying the formation phase of berry development, are limited because of the complex nature of the study and also due to the incorrect assumption that the compounds formed and/or assimilated during this earlier stage are of little importance for the sensorial aspect of grapes and wines [72]. Nevertheless, it is known that berry volume increases during this period and some compounds are accumulated, like tartaric acid and malic acid which are crucial for wine quality. Other compounds that also are accumulated are hydroxycinnamic acids [79] (disseminated in the pulp and skin, important due to their involvement in browning reactions and for being precursors of volatile phenols [80]), monomeric catechins and other tannins [81,82] (tannins are present almost exclusively in the skin and seeds, are responsible for bitterness and astringency, and are also important for colour stability of red wine), minerals [83], amino acids [84], micronutrients and aroma compounds, like methoxypyrazines [85,86]. These compounds are likely to remain in the grape during later periods of ripening (at lower concentrations because of the increased berry volume, dilution) and also at harvest, and might be critical to overall wine quality.

After *veraison*, the berries begin to accumulate sugars (sucrose, and hydrolysing it to glucose and fructose [87]) and malate becomes the carbon source for respiration [88] - sugar concentration at harvest will be dependent on several aspects, like crop load, size of the canopy, health conditions, water conditions and the allowed time period for ripening. Numerous compounds determinants for wine quality are formed during this period, like most of the aroma compounds and aroma precursors (often glycosides) [89,90,91,92,93]. Aroma compounds are distributed between pulp and skin of the berry while they are being formed, being concentrated in the skin in latter stages; anthocyanins are generally concentrated in the skin.

As it is for malic acid, some methoxypyrazines (usually undesirable and causing vegetal, herbaceous notes) tend to decrease their concentration during ripening (it is linked with sunlight exposure of the clusters, and can be manipulated if needed) [94]. Tannins per-berry also decline during ripening - seed tannins decrease due to oxidation as they are fixed to the seed coat, reducing bitterness [95]; skin tannins generally increase their size due to reaction with pectin and anthocyanins, resulting in changes of wine texture and colour stability [81].

2.3.2. Carotenes and chlorophyls

The grapevine response to ultraviolet radiation (UV) depends on the cultivar, the incident UV dose, the ratio of UV and photosynthetically active radiation (PAR) and some environmental conditions [96,97]. Furthermore, different consequences seem to take place depending on the plant organ (leaf, stem or berry) and its developmental stage [97].

The major carotenoids in grapevines are β-carotene and lutein, some times in the range of mg/kg; the residual portion might be present at levels of μg/kg by other xanthophylls, including neoxanthin, violaxanthin, luteoxanthin, lutein-5,6-epoxide and zeaxanthin, and *cis* isomers of lutein and β-carotene [98,99,100]. The carotenoids proposed to be involved in the aroma of wine are β-carotene and neoxanthin. However, lutein and violaxanthin might also be considered because they go through breakdown reactions that may produce norisoprenoid compounds [101].

The carotenoid content in grapes can be influenced by several factors, which include grapevine variety, climate conditions, maturity evolution, soil characteristics and also viticultural practices.

Light is one the environmental factors with the highest influence on the growth and development of plants, through the photosynthesis process. Light is also a main factor responsible for the biosynthesis and regulation of carotenoids [98,101,102].

Generally, the highest carotenoid content in grapevines occurs in hot regions of the world. Temperature has a main effect on grape composition which is a complex process and should be associated with sunlight exposure degree. Apparently, light stimulates the formation of carotenoids in the unripe grapes (before *veraison*), compared with shaded grapes; during ripening, grapes exposed to sunlight display a decrease of carotenoids compared to grapes under shade conditions [98]. Carotenoids are synthesized mostly until *veraison* and then degrade till the end of ripening. The levels of β-carotene, lutein, and neoxanthin decrease between *veraison* and full maturity [99]. This could be related to chemical and enzymatic degradation, or it could be due to mechanisms of conversion in other compounds, like the formation of violaxanthin from β-carotene as a consequence of the activation of the xanthophylls cycle at the end of maturation [98,103]. Carotenoids have higher concentrations in the skin of ripe grapes than in the pulp [103,104]. Differences of carotenoid levels during ripening were described and have been linked with the formation of C_{13}-norisoprenoids (varietal aromas) [98,99,101,105].

β-Carotene and lutein are the most important carotenoids present in the grapes and display different behaviour according to the winemaking process [106]. In 'Vinho do Porto' wines, the profile of carotenoids was similar to the equivalent grapes, even though at lower concentrations. Carotenoid concentration seemed to be dependent on the age of wines, having found lower carotenoid concentrations in aged wines than in younger ones [106,107]. Carotenoid content in red table wines is, normally, inexistent or present in very lower contents. Again, the winemaking process might be the reason for this behaviour: as in port wine the alcoholic fermentation is stopped with the alcohol addition, between the middle to the last third of its length, carotenoid degradation might not be complete, and the added alcohol might help the stability of the present carotenoid molecules. There were not reported carotenoid present in white grape must or wines, probably because the grapes were usually subjected to pressing without skin maceration.

Carotenoid degradation might generate several compounds, some of which are norisoprenoids, which might have impact in wine quality because they have aromatic properties [101,108]. Norisoprenoids have been identified in both white and red wines, from several grape varieties [101,105,109]. It was also reported that β-damascenone and β-ionone might enhance fruity aromas and mask herbaceous notes, having then an indirect influence over the sensorial impact of these wines [110,111].

Carotenoids might degrade either by enzymatic or non-enzymatic reactions, producing norisoprenoids. Norisoprenoids, in other hand, can derive from direct degradation of carotenoids or via glycosylated intermediates [101,108,112]. The norisoprenoids that form the free fraction (non-glycosylated) constitute the C_{13}-norisoprenoid varietal aroma in grapes. The remaining compounds, that are in the bound fraction (glycoconjugate) could remain 'stored' and might be released in the aglycone form during alcoholic fermentation, via enzymatic and acid hydrolysis [101,108,112].

2.3.3 Aroma compound composition

One of the crucial aspects that can help to define wine quality is the aroma. Aroma refers to the detection of volatile compounds using the olfaction receptors (smelling); these compounds were previously liberated by the matrix. Hundreds of compounds are already known to contribute to wine aroma, with impact on the base aroma, contributing to a particular odorant descriptor (ex. citrus aroma, white flower aroma, etc.), contributing to a specific descriptor (ex. banana aroma, rotten egg aroma, etc.),

or by being a specific aroma of a variety or a wine style (ex. green bell pepper aroma, kerosene aroma, etc.) [72]. The impact of the compound to the wine aroma is dependent on the concentration of the individual compound and also dependent on similar chemical-family compounds concentration or similar aroma compounds. For some compounds, different concentrations result in different aroma descriptors.

Aroma compounds of the wine can be divided according to the origin, and be classified as:

Primary aroma compounds, if coming directly from grape composition at the time of picking;

Secondary aroma compounds, if produced by fermentation processes;

Tertiary aroma compounds, if originated during the conservation of the wine until consumption.

The aroma of the grape, composed by a large number of compounds and chemical families, can be used to describe wine quality, the technological potential of the grapevine variety, and also allows to identify grapevine varieties and/or origin of production (when complemented by wine tasting). This could mean that some aromatic components might be used as technological markers - for storage and conservation of wines, for determining the origin and authenticity of protected origin wines, and also contributing for valorisation of minor grapevine varieties and biodiversity preservation [72].

The primary aroma of the wine can also be named ‘varietal aroma’ because the volatile compounds present in the berries give a characteristic and distinctive aroma to the wine, and this aromatic composition is different and distinct to each grapevine variety (‘from the variety’). In fresh grape berries, the large majority of the aroma compounds are present in odourless form, aroma compounds are linked to a sugar molecule - the glycoconjugate forms. The free form of each compound (the aglycone form) is odorous, and is liberated into the wine by acidic and enzymatic hydrolysis. Glycoconjugate hydrolysis is enhanced with the fermentation progress, that is the reason why the grape juice is not particularly aromatic even though the wine can be aromatic. The ‘aromatic’ varieties got this designation because can be described as having large concentrations of free aroma molecules, higher than the threshold of aroma perception - so their berry aroma is intense, differing from ‘neutral’ varieties, which have concentrations of aromatic compounds below the threshold of perception or without any distinctive aroma.

During the ripening phase there is the development of interesting berry flavours as result of the changes in sugar-acid balance, phenols and aroma concentration - this can be named as 'Flavour ripening'; the development of the aromatic portion of the flavour detected by berry tasting is called 'engustment' [113]. One important and distinctive feature of the description of the 'engustment' phenomena is the increase in concentration of the aroma compounds (both in free and glycosylated forms) in the advanced stages of ripening when the sugar increase per berry is already slower [114].

The grape aroma compounds and aroma precursors are produced during the complete berry development and the mixture at each point of the development/ ripening/ harvest depends on several variables, including the environmental conditions during the dormant and growing periods, vineyard and canopy management, degree of ripening and the grapevine variety.

In aromatic varieties, there is high concentration of 'varietal specific' aromatic compounds with an impact above the other classes of aromatic compounds; the concentration of 'varietal' compounds is above its threshold perception level, resulting in highly odorous grape juice. If the variety can be classified as a 'floral' variety, the concentration of monoterpenes should be higher than the rest of the aromatic compounds; if the variety is 'non-floral', the concentration of monoterpenes should be similar to the rest of the aromatic compounds, what might also result in high complexity of aromas [72].

In general, concentration of terpenes and benzene derivatives compounds can be found relatively high in flowers, decreasing during fruit set and increasing again during the ripening phase [115,116]. Usually, the concentration of carotenoids, precursors of C_{13}-norisoprenoids, increases from fruit set until *veraison* and then decreases during ripening, at the time the concentration of C_{13}-norisoprenoids increases [117,118]. Some other classes of aroma compounds, such as methoxypyrazines, accumulate during berry developing, decreasing during ripening, while some other specific compounds, like aliphatic esters and thiols, are synthesized entirely during ripening [119].

In red varieties, the content in varietal volatile compounds is usually high at maturity by sugar/acidity ratio, and remains fairly constant during the subsequent weeks [120,121]. For instance, esters in Cabernet Sauvignon are representative of first stages in berry development, aldehydes of intermediate development stages, and alcohols of the later stages [122]. Adding to this, terpenes are also characteristic of early development (like eucalyptol, β-caryophyllene, and R-humulene), while benzene derivatives tend to appear later (like phenylethanol and 2-phenylethanal) [72,123]. The late prevalence of alcohols is positive because alcohols tend to have higher herbaceous odour thresholds

than aldehydes, and also because of great tendency of alcohols to form fruity-character esters with carboxylic acids.

In white varieties, the evolution of concentrations of volatile compounds during ripening differs with grape variety, and determining maturity using varietal volatiles contents might be more difficult to perform [124] - in Fernão Pires variety [125], C_6-aldehydes, alcohol, norisoprenoids concentrations increase in the three following weeks after *veraison*, and then decrease fast; for several Spanish white varieties [124], C_6-aldehydes and alcohols increase until maturity is achieved, but terpenic compounds increase only until mid-ripening; for Müller-Thurgau, Muscat Ottonel, Gewürztraminer and Kerner varieties [126], there was a substantial loss of free volatile terpenes (FVT) and potentially-volatile terpenes (PVT) between berry harvest and grape pressing stages (at the winery) and the contents were higher 10 to 20 days after the designated date for harvest; in less ripe white varieties [127], it was found higher ester and fatty acids levels and lower concentrations of terpene and benzene derivatives, when compared with mature grapes; for different white varieties [128], monoterpenoids content increased with ripening, sesquiterpenoids and C_{13}-norisoprenoids increased until maturity and then decreased, n-hexanol (herbaceous aroma) decreased gradually and total ester and alcohol content remain similar during the weeks around maturity.

The evolution of volatile compounds during berry development suggests that the dependence on enzyme activity and enzyme specificity to be higher than the dependence on the levels of fatty acid unsaturation [72], so there might be a possibility of using the alcohol/aldehydes ratio for scheduling harvest, aiming to maximize grape and wine aroma.

Several factors affect aroma compound composition and aroma quality of grapes, and each have different impact - knowing how they can affect grape content it is important to orient grape production towards wine production goals. The main factors affecting aroma content and quality are the vineyard site (location, soil, and climate) and vineyard practices (sunlight exposure, canopy training and management, water management, vineyard fertilization and vine disease management).

Vineyard location is critical and restrictive of grape production quality, and cited as the most important factor affecting grape composition and sensory quality, followed by canopy management [129]. It is commonly accepted that restrained grapevine vigour due to local conditions and correct vineyard practices can produce grapes and wines of higher quality. Understanding the influence of the ‘terroir’ (French term that covers topographic, agro-pedological and climatic conditions) can help the grape grower to

produce grapes with the attributes it desires and managing production conditions to achieve the highest wine quality.

The first feature related with vineyard location that favours the production of grapes with higher quality is a limited soil fertility - sufficient nutrient levels limit vegetative growth, creating a situation where a higher proportion of photosynthates might be focused to fruit ripening, favouring aroma and mouth feel formation. High soil porosity is common in low fertility soils, a feature that is also positive because it might create an additional restriction to vegetative growth (due to mild water deficit), might improve microclimate around the vine (favours rapid warming from sun exposure and also rapid irradiation, and minimize frost severity), might have better drainage (promotes early spring growth and limits skin fissures which is good against diseases) [72].

Another feature beneficial for grape quality is medium to low rainfall - although water stress should be avoided during earlier stages of vegetative growth due to high impact in limiting growth, dry conditions enhance disease resistance and usually improve grape ripening (grapevines grow roots deeply so life-threatening conditions due to water stress and nutrient deficiency might be minimized). Nevertheless, water stress at fruit set can be positive because it could limit berry cell division and berry growth, resulting in berries with higher skin/pulp ratio, and subsequent higher concentration of skin products in must and wines - the reduction of cell volume as result of early water deficit is irreversible and the results of having small berries prevail until the end of annual cycle [130].

Temperature during growing season (both vineyard climate, and vine microclimate) is another important feature towards grape quality. It is known that cool conditions help to retain fruit acidity, which enhances colour stability of wines and also microbial stability; temperate conditions seem to benefit formation of grape aroma compounds and their preservation [72]: Grenache wines produced in warmer areas showed to have higher β-damascenone and geraniol concentrations, whereas wines produced in cooler and late ripening areas had lower β-damascenone and higher β-ionone concentrations [131]; Traminette grapes produced higher concentrations of C_6 aldehydes in cooler areas and higher concentrations of monoterpenes in warmer vineyard locations [132].

Altitude of vineyard location influences the temperature and sun exposure of the vines, normally creating conditions for cooler and longer ripening seasons, but also increases the chances of frost damages. Where possible, planting vineyards in locations on slopes or close to large bodies of water can help to avoid these negatives features, always having in mind the number of growing degree days for growing season and the year.

Several authors mentioned that soil depth, drainage and water-retaining capacity are more important factors to wine quality than soil type and composition (far more important than climate and grape variety), however others believe soil can have an independent effect on grape quality [133,134]. As mentioned above, soil characteristics may affect the availability of nutrients and water, can affect vine microclimate through capacity of retaining heat and light reflectance, and root development through its penetrability [133]. Soils with clay composition usually have higher water retention capacity and retain large volumes of water, while sandy soils have better drainage [135,136]. Clay and calcareous clay soils, with good water-retaining capacities and good drainage, may produce grapes that have higher volatile compounds content than in sandy soils. Wines produced by grapes from vineyards with cover crop also show higher volatile compounds content when compared with clean control [137]. Wines from Cabernet Franc, Cabernet Sauvignon and Merlot from vines grown in well-drained gravel soils showed to have lower concentrations than wines from limestone or clay-silt soils [138]. Other research stated that soil type has significantly affect the content of aromas' descriptors of the solvent and the green series if grapes are harvested early, and affect all aroma series except fatty series if harvest occurs at full maturity [139]. Wines produced by grapes from clay soils had higher contributions from floral, sweet and fruity series, while grapes from sandy soils produced wines that have higher attributes of solvent and green series. Wines from potassium-rich soils have higher concentrations of α-ionone and ester acetates (mainly isoamyl acetate) [140]; sparkling wines from calcareous clay soils have higher concentrations of varietal compounds than sandy soils or clay soils; concentrations of monoterpenoids, sesquiterpenoids and C_{13}-norisoprenoids in wines from calcareous clay were higher than wines from clay soils and sandy soils, but total volatile content in clay soil wines is similar to those of calcareous clay soils [128].

Climate and yearly weather are also important factors influencing the aroma compound content and grape quality. Hexanal, (E)-2-hexenal, some carbonyl compounds, (E)-anethole and estragole were identified as the most abundant in less ripe juices [141]. Musts from ripe grapes tended to have higher concentrations of decanoic and dodecanoic acids, and also a larger aliphatic/aromatic ester ratio. The vintage-dependence degree of aliphatic alcohols and terpenoids apparently also depends on variety.

Beside vineyard location, vineyards practices highly affect the development of aroma compounds in several different ways [142] - through the influence over the amount of sunlight that the vine and the clusters are exposed to (light exposure prevents disease incidence, and enhances aromatic and polyphenols development), the total

leaf area and exposed leaf area due to the vine training system (conditioning the ability and efficiency of the vine to perform photosynthesis, regulates bud differentiation, cluster exposure, leaf transpiration), managing the water status of the vine (can restrain vine growth, berry and metabolites development), vine nutrition, particularly nitrogen management (and can have negative impact on aroma of wines) and also fungicide treatments [143].

The effect of sunlight exposure on grape metabolism is complex: direct light can cause dehydration and increased temperature (resulting in water and heat stress) but can also provoke positive changes in photosynthetic pigment content. Light exposure in green berries can increase carotenoid concentration (precursors of C_{13}-norisoprenoids) [144,145], increase concentration of glycosides of terpenols and phenols, and contribute to the reduction of methoxypyrazines concentration (which are sensitive to light) [146,147]. An experiment with Muscat cultivars subject to different sunlight exposures was performed [148]; highest concentration of free terpenols was found for clusters with 50% exposure but the differences relative with 100% exposure clusters were negligible; linalool levels in wines showed to be sensitive to sunlight exposure of respective grapes and this behaviour of monoterpenes was attributed to berry temperature due to sunlight influence. Clusters of Muscat of Frontignan naturally shaded by vine foliage had similar levels of free and glycoconjugates volatiles to those clusters exposed to the sun but clusters shaded by 90% (using shading cloth) had lower concentrations of monoterpenols and C_{13}-norisoprenoids [145]; performing similar study with Syrah, shaded clusters (naturally by vine foliage or by using shading bags) had lower levels of glycoconjugates, particularly phenolic and C_{13}-norisoprenoid glycosides, than clusters exposed to sunlight; 30% or 50% whole vine shading reduced concentrations of glycosides of terpenols, phenols and C_{13}-norisoprenoids, and cluster shading appeared to have greater effect than vine shading [144]. Also with Syrah, glycosides of β-damascenone and 1,6-trimethyl-1,2-dihydronaphthalene (TDN), colour, anthocyanins and tannins decreased in wine due to extreme shading and that shaded berries were rated lower for astringency, fruity flavour and flavour persistence although there was no significant difference in aroma attributes [149].

Vine training and canopy management are influenced by sunlight exposure and several other production variables; many vine training systems were created taking these into account. There are few balanced evaluation studies related vine training systems and canopy management techniques overseeing their influence on grape and wine aroma and flavour composition (balanced conditions should involve the same variety, vine age, canopy management during study period and also over former years, vine nutrition, etc., at least). These studies usually focus on minimizing production

costs or maximizing quantity without loss of quality than focusing on maximizing aroma or solely maximizing quality. In a study comparing several training systems, alternate double crossarm training was referred as producing higher free volatile terpenes (FVT) and potentially-volatile terpenes (PVT) than Lenz Moser, low cordon, low-V and pendelbogen training systems, that removing basal leaves 45 days post-bloom would increase it further [150]. In another study, basal leaf removal also increased FVT and PVT in Gewürztraminer and produced berries richer in muscat and floral aromas than berries from vines that were unhedged or hedged to approximately 14 leaves per shoot [151]. Higher amounts of FVT and PVT were obtained after performing cluster thinning and basal leaf removal compared with only performing thinning or hedging. The highest value was obtained when thinning was performed at *veraison*, wines had more intense floral and muscat scented aromas [152]. Viognier in Smart-Dyson trellising showed to have higher levels of linalool, α-terpineol, β-damascenone and n-hexanol than if trained in vertical shoot positioning (VSP) or Geneva double curtain (GDC) [153]. In some cases, wines from GDC vines had higher concentrations of phenol-free glycosides, with fruitier and floral aromas than those produced using other systems, which could be attributed to GDC allowed greater fruit sunlight interception, higher cluster number and crop yield, and lower cane pruning weight per meter of cordon. In another work, authors have found higher levels of monoterpenes in grapes from VSP training than in Scott Henry, Smart-Dyson, high cordon or GDC training and credited to VSP creating less extreme exposure to sunlight than GDC or high cordon [132]. It was noticed, for Cabernet Sauvignon and Sauvignon Blanc, a combination of leaf thinning and removal of lateral leaves in the fruiting zone could produce higher aromatic ripening [138]. The removal of shoot basal leaf, especially early shoot leaf removal (at pre-flowering, during flowering or just after fruit-set), might have impact the grape aroma composition, increasing the concentration of monoterpenes and principally of C_{13}-norisoprenoids [154].

Water management of the vineyard is another crucial aspect for grape production, directly affecting yield but can also improve wine quality, colour and aroma, if a moderate water deficit occurs [155,156]. Studies usually focused on the evaluation of irrigation needs and irrigation protocols, and findings depended on the studied variety. Non irrigated Cabernet Sauvignon vines (unless water potential dropped below -1.6 MPa) produced grapes with more fruit flavour, with intense red and blackberry, jam or cooked berry and dried fruit aromas, combined with less astringency, bell pepper and black pepper aromas and vegetable notes, than those wines from irrigated vines [157]. Similar results were stated for Cabernet Sauvignon on 1103P and SO4 rootstocks [158]. Sauvignon Blanc vines subjected to moderate water stress produced higher concentrations of cysteinylated aroma compound precursors than those without water

limitation [138]. By other way, water stress should not be higher than mild for obtaining maximum aroma expression in Sauvignon Blanc grapes [159]. Limited water availability for Agiorgitiko may increase glycoconjugates aroma components and the wines are preferred in wine trials panels [160]. In another study, Merlot vines supplied with 35% of estimate crop evapotranspiration during berry development produced higher contents of vitispiranes, β-damascenone, guaiacol, 4-methylguaiacol, 4-ethylguaiacol and 4-vinylguaiacol than irrigated vines, but irrigation deficit had no effect over volatile esters and terpenes concentrations [161]. Wines from Chardonnay irrigated vineyards had more intense aromas of apple, citrus and floral, and less earthy aromas, than un-irrigated controls [162].

In a study about the mechanisms by which water deficit influences the synthesis of aroma compounds [155] also in Cabernet Sauvignon and Chardonnay some contradictory behaviours were found - in Chardonnay, water deficit induced photo-protective mechanisms, activating parts of the phenylpropanoid, energy, carotenoid and isoprenoid pathways that contribute to production of antheraxanthin, flavonols and aroma volatiles, and lowering the concentration of abscisic acid (ABA) following *veraison*; in Cabernet Sauvignon, having anthocyanins, water deficit increased ABA concentrations, and also proline, sugar and anthocyanin concentrations (which were not increased in Chardonnay, suggesting their dependence on ABA). Water deficit increased the transcript abundance of lipoxygenase and hydroperoxide lyase in the fatty metabolism pathway, which is known to affect berry and wine aromas.

Nitrogen fertilization can produce excessive vine vigour, susceptibility to grey rot but also enhance aroma expression. Higher nitrogen supply in Sauvignon Blanc may lead to higher cysteine precursor levels in must, and also higher concentration of glutathione (antioxidant and aroma protective substance) and lower concentrations of phenolic compounds, if fertilization occurs at berry set [163]. Another work [164] mentioned that there was no difference in leaf nitrogen levels when comparing nitrogen application to the soil or foliage, but that the concentrations of volatile thiols, glutathione and varietal aroma intensity were higher if foliar application was used, especially when combined with sulphur. Nitrogen fertilization in the vineyard can produce wines with higher concentrations of 1-butanol, *trans*-3-hexen-1-ol, benzyl alcohol and the majority of esters in Riesling wines, and lower the concentrations of amyl alcohols and 2-phenylalcohol [165].

Finally, disease control substances, especially fungicides are known to affect fermentation kinetics, which eventually affect wine aroma (if disease in the grapes and vines occurs, wine aroma will be also affected).

One important consideration must be in mind related to aroma of wines: how to express the concentration in grapes. From an oenological point of view, it is important to express aroma concentration per berry weight or berry or must volume (by example, using $\mu g\ L^{-1}$) because this parameter is directly correlated with the potential concentration of the substances in wines; from a viticulture point of view, it might be more appropriate and useful to express concentration per single berry (by example, using $g\ berry^{-1}$) in order to avoid the dilution effect due to water or accumulation of soluble solids during ripening.

Some considerations about different classes of aroma compounds will be focussed in these last paragraphs.

The main groups of varietal/primary aroma compounds include terpenes (monoterpenes, sesquiterpenes and C_{13}-norisoprenoids), shikimate pathway derivatives (volatile phenols or benzene derivatives), aliphatic C_6 volatile compounds (aldehydes and alcohols), volatile thiols (mercaptan derivatives) and methoxypyrazines.

Terpenes are a large group of organic compounds produced by plants, and are the main components of essential oils; terpenes are isoprenoids, derived from a 5-carbon unit with C_5H_8 formula (isoprenic unit); terpenes exist as multiples of the isoprenic unit: hemiterpenes (with 5 carbons; C_5), monoterpenes (C_{10}) and sesquiterpenes (C_{15}), and diterpenes (C_{20}, which are still volatile under normal atmospheric conditions) [166]. The most predominant group in grapes and wines are the monoterpenes ($C_{10}H_{16}$) and more than 50 terpenes have already been identified in grapes and wines - linalool, geraniol and nerol are the most common terpenes present in grapes and the typical aroma descriptors include floral, rose, citrus, coriander and spicy aroma. Terpenes are volatile molecules present in white varieties like Muscats or Gewürztraminer, but they are also present in red varieties like Touriga Nacional [167]. The most common monoterpenes present in must and wines are linalool and its related compounds (pyranic and furanic oxides, linalool hydroxides, diols), geraniol and its related compounds (*iso*geraniol, nerol, nerol oxide, rose oxide), terpineol and citronellol [168]. While their aroma descriptors are attractive in concentrations usually present in wines (rose, camphor, coriander or citric notes), when monoterpenes are present in high concentration they can become undesirable due to their herbaceous notes. Sesquiterpenes are present in grapes as free forms: farnesol is one of the most common, and others like rotundone, α-caryophyllene, α-copaene, α-cubebene, muurolene, calamenene have been already detected in several grape varieties.

Norisoprenoids are originated from oxidative degradation of carotenoids (C_{40}, tetraterpenes, like β-carotene and lutein) and the most abundant in grapes are the C_{13}-norisoprenoids. C_{13}-norisoprenoids are present as glycoside compounds and their aroma descriptors are floral, fruity and spicy, and they have special impact in neutral varieties like Sangiovese [72]. C_{13}-norisoprenoids are classified in megastigmanes (oxygenated on carbon 7 - damascene group - or in carbon 9 - ionone group) and non-megastigmanes. Megastigmane group compounds include β-damascenone (aroma described as fruity-floral, honey-like and stewed apple notes, and mentioned often as an 'aroma booster'), 3-hydroxy-β-damascone (tobacco characters), β-damascone (fruity and tobacco notes) and 3-oxo-α-ionol (tobacco characters) and β-ionone (aroma described as sweet and violet notes), and they have very low aroma threshold (in ng L^{-1} range). The presence of some carotenoids can influence the perception of other compounds - it was cited that β-damascenone has an indirect impact on red wine aroma because it increases the threshold of fruity esters, like ethyl cinnamate or ethyl caproate, and decrease the odour threshold of green bell pepper notes [169]. As for the non-megastigmanes, the most known are 1,6-trimethyl-1,2-dihydronaphthalene (TDN), (E)-1-(2,3,6-trimethylphenyl)buta-1,3-diene (TPB) and some actinidols. TDN aroma can be described as a kerosene-like character (can be positive or negative, depending on the wine and varieties), while TPB, when present in high concentrations, can contribute to floral, geranium, and insecticide or plastic character of wines. Carotenoids and norisoprenoids tend to accumulate during ripening and break-down into smaller compounds when grapes reach maturity phase - in grapes they exist bounded to sugars, being released during alcoholic fermentation (becoming aromatically active). Carotenoids are formed in berry skin, being increased with higher sunlight exposure because carotenoids function is to protect grape tissues from sunlight and ultraviolet light [170].

Benzene derivatives are another important group of varietal aroma compounds, and include molecules such as benzyl alcohol, vanillin, methyl vanillate, acetovanillone and homovanillic alcohol. The typical descriptors associated with benzenoids are spicy, tobacco, citrus, honey, vanilla and floral, and become with undesirable notes at high concentrations, described as chemical or phenolic - these compounds are present in free and glycosylated forms, and aroma thresholds vary between μL^{-1} and 10 mgL^{-1}.

Thiols are compounds containing sulphur atoms, analogue of an alcohol (as mercaptans) are responsible for high impact wine aromas, pleasant but also undesirable - they are present in grapes as cysteine and glutathione S-conjugates exclusively. Most important volatile thiols identified in grapes are 3-mercaptohexanol

(3MH - passion fruit and grapefruit notes), 3-mercaptohexyl acetate (3MHA - boxwood, grapefruit peel and passion fruit characters), 4-mercapto-4-methylpentan-2-one (4MMP - boxwood and broom descriptors), and 4-mercapto-4-methylpentan-2-ol (4-MMPOH - citrus peel characters) - these compounds have a positive impact in wine aroma [138,171,172,173]. Some other compounds have their origin in fermentation process and also contribute to the final wine aroma, for example, hydrogen sulphide (H_2S) that has a negative impact in the wine aroma, due to rotten egg aroma, are formed by yeast during fermentation - this aroma is common, especially when alcoholic fermentation occurs in difficult conditions and yeast struggles to complete fermentation (low nutrients, high alcohol, low oxygen content, reductive conditions, etc.). Other typical off-flavours of thiols are cooked vegetables, onion, plastic, Band-Aid or cabbage, and are formed by compounds such as thioacetic acid esters or other mercaptans [174]. Thiols are present in varieties like Sauvignon Blanc, Riesling, Colombard, Semillon, Cabernet Sauvignon and Merlot [171], existing in traces in grapes and are formed during alcoholic fermentation, through mechanisms not yet fully understood.

Methoxypyrazines are odoriferous compounds that contain nitrogen and have been identified in several different varieties such as Sauvignon Blanc, Cabernet Sauvignon, Cabernet Franc, Merlot and Semillon; they contribute with aromatic notes like vegetative, herbaceous, bell pepper and earthy, so they can have positive impact but also negative, depending on the concentration. The most important methoxypyrazines identified in wines are 2-methoxy-3-isobutylpyrazine (IBMP - green capsicum character), 2-methoxy-3-isopropylpyrazine (IPMP - asparagus or sweet pea notes), and 2-methoxy-3-sec-butylpyrazine (SBMP - galbanum oil characters) [175].

Higher alcohols are alcohols that have more than 2 carbon atoms and so they have higher molecular weight and boiling points than ethanol. Higher alcohols are present in wine, formed by yeasts during alcoholic fermentation and they can be produced from sugars or from amino acids (the amount of formed higher alcohols depend of several aspects such as genus, specie and strain of yeast, nutrients and composition of the must, temperature, aeration and pH during fermentation). Higher alcohols can be related with the amino acid from which they were produced - leucine with 3-methylbutanol, isoleucine with 2-methylbutanol, valine with 2-methylpropanol, threonine with propanol and phenylalanine with 2-phenylethanol. The impact of the presence of higher alcohols can be either positive or negative. Because of the high odour threshold and low concentration present in wines, the impact of higher alcohols can be small or inexistent; their impact is more significant in distilled beverages because they are present in higher concentrations. As example, *iso*amyl alcohol is one of the major higher alcohols present in wines, can be described as banana character.

Aldehydes and aliphatic alcohols compounds with 6 carbons already identified in wines can be described as having herbaceous, unripe fruit and crumpled leaf notes, so their presence is negative to wine's aroma. The major aliphatic alcohol present in grapes is hexanol, Z-3-hexenol and E-2-hexenol, and hexanal, Z-3-hexenal and E-2-hexenal are present in grapes in lower amounts.

Volatile phenols are compounds with high impact in wine aroma; the major phenols found in wines are 4-ethylguaiacol (4-EG), 4-ethylphenol (4-EP), 4-methylguaiacol, vinylphenols, guaiacol, eugenol, and vanillin. These compounds can have positive impact in wine aroma when in low concentrations and become off-flavours when present in higher concentrations. The common descriptors go from sweaty saddle, leather to cloves. In any case, because of their low thresholds, a small concentration can produce great aromatic impact and completely overwhelm the aroma of the wine. The presence of these compounds in wine can be explained to one of three origins: microbial, oak maturation and smoke-taint [176,177,178].

2.3.4 Composition of Baga grapes and wines

Unfortunately, there are just a few publications regarding Baga varietal aroma and wine chemical composition, especially when Baga represents a large percentage of the total area of red varieties planted in Bairrada region. In addition, none of the papers reported in literature has sensorial data analysis of Baga wines.

A first publication found in literature [179], with the aim of performing the aromatic analysis of Baga red wines and to identify possible impact odorants, was based on a liquid-liquid continuous extraction method with dichloromethane followed by analysis by gas chromatography-mass spectrometry (GC-MS) and subsequent identification of impact odorants by calculating aroma index using odour thresholds from literature. A total of 53 compounds were identified and quantified, with the majority being aliphatic and aromatic alcohols (44%), acids (27%), esters (15%), and small quantities of lactones (6%), amides (5%) and phenols (1%). Nine compounds were determined to be the odorants with higher impact: guaiacol, 3-methylbutanoic acid, 4-ethoxycarbonyl-γ-butyrolactone, *iso*butyric acid, 2-phenylethanol, γ-nonalactone, octanoic acid, ethyl octanoate and 4-(1-hydroxyethyl)-γ-butyrolactone. Another research work focused on the evolution of varietal aroma of Baga variety during ripening [180]. It was reported a ripening, since *veraison* until full ripening, using headspace-solid phase microextraction technique (HS-SPME) and a GC-MS quantification methodology. A large number of sesquiterpenoids, monoterpenoids and norisoprenoids were identified in samples

during the ripening from two different vineyards and the majority of compounds found were obtained at full technical maturity (determined by sugar acid ratio). The authors concluded that sesquiterpenoids were an important group for varietal aroma of Baga.

Apart from these papers, little information regarding Baga aromatic composition was found in literature. Another important aspect to be mentioned is that it is regionally accepted that Baga wines need some time in wood barrel before being ready for consumption, so it was difficult to find Baga wines commercially available that did not overpassed barrel ageing [181].

2.4 Yield control techniques

In years of excessive production or for certain grape varieties, yield control is an important issue, as consequence of using better plant material (obtained from clonal selection). Using better plant material, even though presenting clearly advantages, is sometimes perverse and might lead to excessive grape production. Nevertheless, yield control is an old problem in viticulture.

A grape producer that intends to obtain yield control can use one or more of the following yield control strategies, divided into 3 major groups: Long term methods (that relate to vineyard selection and planting), use of some kind of products or mixture, and soil or canopy management techniques.

Vineyard establishment, long term methods and strategies are not usually referred as yield control strategies. Nevertheless, some can be used during the lifetime of a vineyard even though the costs of these methods are generally much higher than the rest the strategies to be referred below. These strategies are related to vineyard site selection and/or vineyard planting, and linked to plant vigor. To name just a few examples, there are the choice (when possible) of the vineyard location, the soil characteristics (fertility, depth, rock composition), sun exposure, water availability, altitude, and slope [182]. Prospect yields are also dependent on the selected rootstock and selected variety clone, row orientation and density of plantation. These kinds of strategies always involve long term decisions and are always the last choice due to high costs; any changes introduced can result in a breakdown of the grape production for a vintage or more.

Soil management techniques can also influence yield in the same harvest, or for several harvests. These techniques include soil nutrition, soil cover cultures, root pruning, and graft-clone selection (by selecting the lower yield graft-variety binary, and it can be changed during vineyard production life, for instance, using T-budding technique [183]. For soil nutrition and cover cultures, the crucial aspect is to maintain balance between the vines necessities and other crops, preventing crop competition, but controlling the yield levels [184,185].

Several studies of performing chemical thinning were made earlier in the twentieth century to find an economical and effective alternative to manual thinning, and continued up to the present. Several compounds were tested, most of them with little success or, at least, too many technical difficulties to be used by wine companies:

Alpha-naphtaleneacetic acid (NAA) [186,187];

Dinitro-sec-butylphel (ND-289) [186];

Gibberellic acid (GA) [188,189,190];

Ethephon, Ethrel (2-Chloroethylphoshonic acid - CEPA) [191,192];

5-Chloro-6-ethoxycarbonylmethoxy-2,1,3 benzothiadiazole (TH 6241) [191];

Denapon (1-naphthyl N-methylcarbamate) [192];

Chlormequat (2-chloroethyl)trimethylammonium chloride [193];

Calcium Prohexadione [194,195].

Chemical thinning usually involved spraying the substance during one or more stages of flower and/or fruit development - the active substance interferes with the flower and/or fruit development and causes the formation of less grapes berries or the abortion of fruits. If the substance is sprayed post-fruit set, it can create no result. In other cases, when using calcium prohexadione, the active substance has an effect in reducing vegetative development of the plant, the vigor and the need of shoot trimming, for example [194].

When applied in earlier stages, this technique requires lower concentrations of active substance to be successful but, at these earlier stages, the leaves/shoots are more susceptive to damage and it is more difficult to predict the need of chemical thinning. If the concentration of the active substance or the amount of product used is too high, a significant decrease of yield can occur.

Other substances, such as TH 6241, can act as plant regulator inhibiting or stimulating ethylene production and cause fruit abscission in nearly mature fruits [191].

Although most of the active substances would successfully cause a reduction of the formed fruits and/or yield, and could decrease berry size and cluster compactness (reducing then the probability of rot occurrence), these techniques were challenging to be established as vineyard techniques and became uncommon: chemical thinning fruit - yield reduction results and fruit quality are difficult to predict, which, associated to difficulties to implement because of the product concentration and spraying calibrations, canopy density, wind and rain conditions close to spraying dates, lead to chemical thinning to become an unusual technique in grape production for wine.

Flower thinning consists on removing flowers from clusters close to bloom [188]. This technique can be performed manually or mechanically, and published results show that, although yield can decrease using flower thinning, fruit set of the remaining clusters usually increase, increasing also cluster compactness (resulting in higher risk of cluster rot presence) [188,196]. It is believed that the plant adapts to lower cluster number and increases the fruit set of the remaining clusters, adjusting the source/sink

ratio - this adjustment can result in difficulties in forecasting the harvest yield. Flower thinning technique can be time and cost-effective if mechanically performed, but it may not answer the concerns about future grape quality and yield forecast.

Berry thinning [197] consists of removing the tips of all clusters immediately after flowering in order to obtain rounder clusters, and is based on the principle that the tip of the cluster matures slower than the rest of the cluster [198,199]; however, little information about the influence of berry thinning on the quality of grapes and wines is available, only has been studied in table grapes. Horizontal division consists in removing manually part of the cluster aiming to produce high quality grapes, focusing on the reduction of disease incidence while producing healthy and fully mature grapes, in moderate yields [200]. Initial trials were only partial [201,202,203] and little is known about intervention timing and the influence on different varieties. An example could be a work that studied this practice in Pinot Gris and Riesling [200]. In this work the authors focused the impact of timing of the cluster division on rot incidence and harvest parameters. These techniques require expertise, are time consuming and expensive, having the positive aspect of removing damaged berries, or green berries.

Shading is a common phenomenon in dense canopies, and creates several problems like an incomplete ripening (low sugar, high acidity), low coloured berries, and development of herbaceous aromas [204]. Light suppression reduces the activity of the plant, whether shading occurs only in the clusters area or in all vine foliage [205]. When light suppression occurs during flowering it can reduce fruit set due to photosynthesis reduction, and this outcome can be used as yield control technique [206]. Intentionally or not, light reduction can be obtained using adjacent foliage (especially when in dense canopies), by boxes or small bags (for individual clusters) or opaque nets placed close to the vines or above the vines. When early severe defoliation may not be suited for dry and hot regions because it may expose the clusters to excessive sunlight (can cause cluster sunburn, severe berry dehydration, low colour and low acidity), temporary shading might be used as a yield control method, acting between pre-bloom and fruit set [206]. This technique could be advantageous as it requires less labour, by avoiding removing leaves. Results obtained showed that cluster compactness, fruit set, berry number per cluster, cluster weight and yield per vine were reduced by using shading techniques, without affecting berry composition. It was reported in literature [207] that shaded clusters showed lower levels of glycoconjugates, particularly phenolic and C_{13}-norisoprenoidic glycosides; for the shaded vines, lower contents of glycosides of terpenols, phenols and C_{13}-norisoprenoids were found.

Cluster shading affected more the berry composition than vine shading. One of the more interesting features of this technique is that is simple and temporary: as long as the shading remains, photosynthesis continues to be repressed but plant goes back to full activity when sun exposure is restored.

One of the most recent alternatives of reducing yield is the application of anti-transpirant at early season, to create a limitation during fruit set and berry development [208] - the possibility to create temporary source limitation by using an anti-transpirant to reduce transpiration and photosynthesis followed similar research data for several other crops [209,210,211]. It was used the anti-transpirant Vapor Gard® (Di-1-p-Menthene, Intrachem Bio Italia, Grassobbio, BG, Italy) with encouraging results as leaf assimilation and transpiration were reduced for several weeks after product spraying in cultivars Sangiovese and Ciliegiolo, compared with control vines. Results showed lower yield, berry weight, berry size, bunch compactness, and also higher sugar and anthocyanin contents. With another aim, the same anti-transpirant, Di-1-p-Menthene, was used in Sangiovese vines post-*veraison* (around 14-15º Brix) with the aim of delaying ripening and reducing sugar accumulation in the berry [212]. Another study was performed using anti-transpirant before bloom and achieved reductions net assimilation in treated leaves for 20 to 40 days after spraying [213]; berry set, cluster weight and yield were significantly reduced, and bunched compactness had a small reduction; soluble solids and titratable acidity showed no significant differences. In another work, a kaolin-based foliar reflective film (Surround WP; NovaSource, Phoenix, AZ) was used to study the effect on the ratio of anthocyanins to soluble solids in deficit-irrigated Merlot grapevines over a 5-year period and it was found that sprayed vines showed a reduced number of berries per cluster [214], a result that was not reported in other former papers [215,216] studying different cultivars. The author refers [214] that the reasons for the observed reduction of the number of berries are uncertain, but the spraying may induce fruit abscission by the presence of the product, by the force of the spraying, or the berries formation was vulnerable at fruit set. However, yield was not affected due to compensation by berry weight increase.

Irrigation might also be used as a strategy to reduce yield, and to achieve better quality. Where irrigation is frequently needed to surpass excessive water stress levels, controlling water stress and limiting the available amount of water could be useful and crucial for limiting yield - as mentioned above, water stress during earlier stages of vegetative growth may be limiting in vegetative growth, especially by limiting the berry growth irreversibly and thus indirectly reducing yield [130]. Higher water deficit introduced

between flowering and *veraison* may lead to lighter berries when comparing to berries resulting from fully irrigated regimens [217]. Some interesting aspects linking irrigation and yield loss or control were found: Regulated Deficit Irrigation (RDI) differences lower than 40% between crop evapotranspiration (ET_O) do not alter yield or quality parameters significantly but reduced leaf growth [218]; significant yield loss was mentioned when using sustained deficit irrigation [219]; two RDI regimens were proposed, both resulting in significant lower yields when comparing with fully irrigating vines [220]; early water deficit at flowering results in poor berry set or aborted grape yield reduction [221]. Irrigation can be used for reducing yield but only when irrigation is needed and only by changing the irrigation regimen to other regimen.

Shoot trimming is a canopy management technique commonly used to prevent diseases and to facilitate the harvests tasks [182,222]. When practiced at certain timings, limiting yield might also be a consequence of shoot trimming, as several works mention. It was referred that using shoot trimming could result in yield loss, increased soluble solids, increased berry anthocyanins but did not alter wine aroma global perception [223]. In some cases, post-*veraison* shoot trimming significantly reduced yield per vine and light trimming had higher reduction effect than severe trimming, probably due to compensation of the plant [224]. The trimming regimens also achieved reduced cluster weight and compactness, lower Brix and pH, and introduced slight effects on titratable acidity, yeast assimilable nitrogen, and total anthocyanins. Some authors referred that shoot trimming has no usefulness before flowering [182], and only has function between the period of 3 weeks after fruit set and the harvest; before flowering, it may reduce the photosynthetic capacity of the plant due to lowering the photoactive leaves; between flowering and 2 weeks after fruit set, it may result in lower fruit set due to lower photoassimilates and also result a stimulate formation of lateral leaves; this lower fruit set can be interesting if lower yield is desired. However, others have not found difference between trimming and not trimmed vines [222], with Touriga Nacional.

Leaf removal is a common practice in vineyards, aiming to control diseases, increase fruit quality and to help in harvest tasks; however it is not always correctly used [182]. Leaves are crucial to produce photoassimilates and removing them provokes a decrease on the overall photosynthetic capacity of the plant; nonetheless, it can improve the sunlight exposure, the microclimate close to the fruit zone and can also increase the efficiency of the disease control products application. When leaf removal is executed manually, is time consuming and can represent high costs; when performed mechanically, it can be faster but care is needed to avoid shoots and vine

damage. Leaf removal alters source/sink ratio, the light and temperature microclimate of the vine, and also alters the leaf age average; it can be implemented from bloom until harvest, producing different results. Several authors mentioned that leaf removal may increase leaf photosynthetic activity [225,226], probably to compensate source/sink ration, but others stated the opposite [227,228]. Additionally, it was also referred that chlorophyll concentration increased after leaf removal [229]. Regarding the vegetative growth and vigor, some variable results can be mentioned: lateral leaves growth was stimulated [228]; leaf removal could cause the vine not to have enough accumulated photoassimilates for the dormant season due to insufficient source/sink ration [230], but also the contrary was affirmed [231,232]; and finally, does not affect pruning weight [226,233]. As for yield, leaf removal effects depend on timing and intensity. Leaf removal between bloom and *veraison* can increase light microclimate and next year fertility [234,235] and may not increase bud differentiation if performed after *veraison* [227]; if leaf removal is executed between bloom and fruit set can result in reduced growth and lower yield [225,230] and, additionally, if performed at bloom it may result in 50% abscission of flowers but only in 25% abscission if performed two weeks after bloom [230]. As some authors reported, performing leaf removal before bloom could cause low yield [236], this was the starting point of studying early leaf removal as a yield control technique. It was used with that purpose [228], obtaining low fruit set, low berry number, low berry weight and low cluster weight; some authors mentioned that may not reduce yield if performed after fruit set [226,234,237,238]; It was also mentioned that high intensity leaf removal could affect flowering, cluster and berry weight in the following year [239] - some other authors reported not to have significant impact on following year [226,237,238,240,241,242]. From all data obtained from early leaf removal, the most important and interesting are the following: leaf removal increases light in the cluster area [227,229,243] (better and more light, and also heat, changes primary and secondary metabolism, like phytocrome reactions that are responsible for anthocyanins and phenolics biosynthesis [244,245]); less leaves may decrease sugar content in berries due to low or insufficient photosynthesis for a correct ripening [246,247] but other authors also mentioned that there was no change in sugar content [226,242,248] and other mentioned higher sugar content [228,249,250]; higher phenolics content [226,228,248,251,252,253], attributed to light exposure, but other did not [233]; lower acidity [254,255], higher anthocyanins content [239,256] attributed to the temperature, and sunburn [257], associated with low aroma and low acidity. It was also reported that higher temperatures in the berries could result in lower anthocyanins contents [239,255] , either by degradation or by inhibition of the biosynthesis, or by both simultaneously. The effect of early leaf removal on the grape aroma is poorly known - while light affects enzymatic activity needed to the formation of aroma compounds, temperature also

affects it, and so moderate exposure might be positive for aroma formation [257]; leaf removal may also result in glycosidic precursors enhancement [248,258] and improving floral aromas while reducing green and herbaceous notes [249]. Early leaf removal promotes better cluster light exposure, better wind exposure (allowing clusters and cluster zone leaves to dry faster), allow to use less disease control products and to make easier applications, induces less *Botrytis* incidence due to better microclimate conditions and due to thickening of berry skin as a result of early light exposure of the clusters [259]. Timing and the severity of the leaf removal are crucial parameters that contribute to the success of this technique, and it is needed to take into account that total leaf area is not the same as exposed leaf area when deciding the severity of the defoliation.

Manual bunch thinning (MBT), or cluster thinning, or fruit thinning consists in removing one or more bunches/clusters from each vine with the purpose of gaining fruit quality, and sometimes, and/or reducing yield. This is one of the oldest methods for yield control, and thoroughly studied. Bunch thinning does not always produces clear and significant results [260], although other authors mentioned that is a useful technique to achieve yield reduction and quality improvement [261,262,263], especially for high-yielding varieties, because it results in a better balanced vine, and can also accelerate ripening 5 to 10 days [264]. Commonly, the cluster removal is performed manually, which is time consuming and represents expensive human labor. The amount of clusters to be removed needs to be established depending of the aims: as examples, it can be removed all green or partially ripe clusters, in order to have more homogenous ripening and better ripeness; it can be decided to have a certain number of clusters per vine so it will be removed all cluster needed to achieve that number; it can be decided to have one cluster per shoot, so it will be removed all the extra cluster from every shoot. Some authors advised to carry out yield estimation and quantification of vine leaf areas prior to perform bunch thinning [265]; others added that over production should be clearly defined to help to decide if bunch thinning is needed [262]. It is frequently argued that high-yielding varieties in a high-yield vintage should not be submitted to bunch thinning because the hypothetical quality increase may be very small and it will not reward bunch thinning costs. Some criteria for selecting the clusters to be removed were defined in literature [266]: disease clusters; late or delayed ripening clusters; clusters higher in the shoot; clusters with more shaded hours. Bunch thinning should be performed at *veraison*, having the risk that the plant might compensate if performed earlier [264]; if executed later than *veraison*, it may only result in lower yield [267]. For some authors [268,269,270] bunch thinning performed at fruit set produced better results,

even though the risk of the plant compensation or the risk of occurrence of climate incidents that could result in loss of production [271], while others [272] referred that results depend more on thinning intensity than on phenological stage when is performed. There are some contradictory results regarding the outcomes of using bunch thinning and the intensity that should be used: the most common bunch thinning intensities between 30 to 60%, some referring 50% [264,267,271,272,273,277] others 30 to 45% [268,274], and some 25% [270] and 60% [275]. The intensity of bunch thinning is not proportional with yield loss [277], and this may be due to compensation of the plant, that can also affect sugar concentration and ripening [276], and successive application of higher bunch thinning severities may reduce its effect in subsequent vintages. In other hand, complete bunch thinning increased plant vigor, shoot weight and shoot diameter [277] what can be important during the formation of a new plant.

One of the most innovative and yet less studied is '*Double Maturation Raisonnée*' (DMR), or '*Passerillage sur souche*', or 'Reasoned Double Ripening'. DMR has being developed rather slowly since the first works [278], and being introduced to several countries to autochthonous varieties. DMR consists on disconnecting from the vine part of the clusters, leaving them hanging attached to the vine wires and exposed to sunlight to dehydrate. The separation by cutting can involve the cluster and/or the shoots; the proportion of clusters to be separated must be carefully selected, as well as the timing of the separation and the number of days between the separation and the actual picking of these cluster - the cutting can be made with the berries unripe, close to full maturity, fully matured, and over ripped, and the period of time of dehydration can go up to several weeks. Consequently, the result is having two different sorts of clusters, with different ripening processes, and so it explains the name '*double maturation*', which means 'dual ripening'. One of the sets of clusters, connected to the vine until the harvest date, can be compared with the clusters from common vines with active ripening [279]; the second set is of clusters separated from the vine, where some degree of dehydration occurs. Depending on the date of the harvest, the first set may be characterized by the same physiological and biochemical reactions and composition as normal grapes [280]. These authors referred also that significant changes occur in the clusters submitted to dehydration, related to the water loss [281] (increase of sugar concentration, for example) and also that titratable acidity remains high due to the end of acid catabolism, lower occurrence of rot, and more 'maturity' in polyphenols [282]. The common results attributed to DMR are lower yield, higher sugar concentration, higher acidity of the must, and low *Botrytis* incidence [279,280,283,284,285]. In most cases, two weeks between cutting the shoots or clusters were sufficient to obtain the appropriate

over-ripening for the harvest [286], but is advisable to proceed carefully to dehydration assessment of the grapes; they mentioned also that shoot cut is better to be performed before complete berry ripening and softening because it enhances grape health (less harm from bee and wasps), since it requires less time to execute when compared with a late shoot cut that may include damaged berries to remove from the vine and finally it may achieve a better balance between both ripening sets. Sensorial scores of wines from DMR were reported to be higher than wines from traditional procedures [283], or, at least, similar [280]. In another work [284] it was reported that scores from DMR wines were higher than traditional wines, and the highest scores were obtained from wines combining DMR with a late harvest date.

One of the major concerns using this technique is the time consuming and personal expertise requirements. Other concerns are the suitability of this technique to different wine styles and the consequences in plant development during the following seasons.

III Material and Methods

3.1 Location and description of vineyard

The trials were installed in a commercial vineyard planted with Baga (*Vitis vinifera* L.), in a property owned by "Caves Messias", during the 2011, 2012, and 2013 growing seasons. The vineyard is called ‘Quinta do Valdoeiro’ (Figure 5 and Figure 6), located in Bairrada region, at Vacariça, closed to Mealhada (40°21'23.55"N; 8°24'58.94"W, altitude between 80 and 100 meters).

Figure 5 - ‘Quinta do Valdoeiro’ vineyard (Vacariça, Mealhada). Aerial photo from Google maps.

The trials were conducted with 15 years-old grapevines cv. Baga variety grafted on 3309 C rootstock, vertically shoot positioned and pruned in Double Guyot, with two pairs of movable wires, planted 1.25 x 2.40 meters, oriented north-south (Figure 7).

Figure 6 - 'Quinta do Valdoeiro' vineyard, with Baga in the foreground, with the markings of the experimental plot (Vacariça, Mealhada).

The Guyot training system is a part of a group of systems referred to as a VSP system - Vertical Shoot Positioned. The training system was intended for low to moderate vigour vineyards - it has fewer buds and vine growth per linear meter of vineyard row than other training systems. Vigorous vines might not be well adequate to the Guyot system, and can result in high shoot density and inner-canopy shading [183].

The soils can be described as having low fertility, poor, consisting of a first sandy layer and another layer, deeper, of clayey consistency, compact, where the roots of the vines penetrate with difficulty; the vineyard is composed by several wave like slopes facing the South and East, with the different varieties separated by plots; all the property is managed according with Integrated Pest Management protocols and only use approved products.

All vineyard practices and treatments were carried by 'Caves Messias' according to the company decisions regarding the plant and grape production needs, excluding defoliation, bunch thinning and DMR.

Figure 7 - Baga in Double Guyot, experimental plot (Vacariça, Mealhada).

3.2 Climate description

Meteorological description was performed for the experimental study, 2011, 2012 and 2013 seasons. Data from these vintages was collected from private meteorological stations close to Mealhada (Cantanhede, 11km and Coimbra, 24 km, respectively), combining values when some was missing. The recorded parameters were air temperature, precipitation, humidity and wind speed (Table 9).

Several bioclimatic indexes were also calculated aiming to know the annual climate variations, as it is shown in Table 14. Meteorological data corresponding to the vegetative season included data from 1 March until 30 September for each year of study. The following classes were defined by the respective authors of each bioclimatic index:

-for Winkler Index [287] - Region I (1111-1390 growing-degree days - GDD) and Region II (1391-1670 GDD) generally produce the best dry table wines with light to medium body and good balance; Region III (1671-1950 GDD) produces full-bodied dry and sweet wines; Region IV (1951-2220 GDD) is best for fortified wines, with table wines being inferior; Region V (2220-2499 GDD) is best for table grapes and makes low-quality table wines;

- for Huglin Index [288] - Unsuitably Cool: < 900; Too Cool: 900-1200; Very Cool: 1200-1500; Cool: 1500-1800; Temperate: 1800-2100; Warm/Temperate: 2100-2400; Warm: 2400-2700; Very Warm: 2700-3000; Too Hot: > 3000;

- for Cool Night Index [289] - Very cool nights: < 12; Cool nights: 12-14; Temperate nights: 14-18; Warm nights: > 18;

- for Branas Heliothermic Index [290] - Unsuitable below 2.6;

- for Hydrothermic Index [291] - Low risk of contamination: < 2500; Medium risk of contamination: 2500-5100; High risk of contamination: 5100-7500; Very high risk of contamination: > 7500;

- for Selianinov Index - Insufficient hydric regime: < 1; Normal hydric regime: 1-3; Excessive hydric regime: > 3;

- and for Growing Season Temperature - Too Cool: <1111; Cool: 1111-1389; Temperate: 1389-1667; Temperate/Warm: 1667-1944; Warm: 1944-2222; Very warm: 2222-2500; Too Hot: > 2500-2778.

Table 9 - Climate description of the vineyard region for 2011 - 2013 vintages (data from Coimbra).

2011	Av. Tmin (º C)	Av. Tmax (º C)	Tot. P (mm)	Av. Hum (%)	Av.Wind (km/h)
Jan.	6.7	13.5	90.5	85.3	9.3
Feb.	6.9	15.5	103.8	85.1	9.5
Mar.	8.3	16.5	54.3	74.1	2.1
Apr	12.8	23.8	56.6	68.9	2.2
May	13.8	24.6	90.3	76.8	2.1
Jun	13.7	25.8	0.2	68.3	2.6
Jul	14.2	26.7	3.2	73.6	4.8
Aug	15.5	27.5	10.6	79.0	2.3
Sep	14.4	27.1	23.6	79.2	1.9
Oct	14.2	26.9	45.9	60.7	2.6
Nov	9.4	16.9	176.0	86.0	2.8
Dec	6.5	13.9	58.6	86.1	2.0

2012	Av. Tmin (º C)	Av. Tmax (º C)	Tot. P (mm)	Av. Hum (%)	Av.Wind (km/h)
Jan.	5.3	13.8	15.8	82.3	1.9
Feb.	3.6	14.6	4.7	65.3	2.2
Mar.	8.9	20.1	14.2	63.8	2.0
Apr	8.5	15.8	113.1	82.8	2.5
May	12.3	22.4	83.9	77.8	2.5
Jun	14.2	25.4	21.4	76.5	2.3
Jul	14.4	26.7	4.0	76.0	9.0
Aug	15.0	27.4	18.5	78.1	8.0
Sep	16.2	28.5	49.6	67.6	6.4
Oct	12.4	21.2	121.2	83.3	8.1
Nov	8.1	15.4	98.4	85.3	8.3
Dec	7.7	14.0	92.3	89.5	9.9

2013	Av. Tmin (º C)	Av. Tmax (º C)	Tot. P (mm)	Av. Hum (%)	Av.Wind (km/h)
Jan.	7.2	13.4	134.1	88.8	8.9
Feb.	4.4	15.0	65.1	89.1	11.7
Mar.	8.4	15.0	239.5	88.2	11.4
Apr	8.7	18.7	71.5	79.5	10.1
May	9.5	20.4	47.6	82.1	2.3
Jun	14.0	24.4	56.6	78.2	9.1
Jul	16.9	29.4	4.3	85.3	4.4
Aug	15.6	30.1	1.1	78.5	7.5
Sep	15.5	29.5	48.8	77.3	8.5
Oct	13.9	21.6	145.5	92.1	8.3
Nov	8.1	15.9	23.2	84.8	8.1
Dec	6.2	14.5	169.4	83.8	10.3

As it can be seen from the data (Table 9), the three years of the study had different weather: 2011 was a warmer year, and rained less during the growing season, although having a good volume of rain during dormant season; 2012 was cooler year, with a dry dormant season but a wet growing season; 2013 was also a cool year, with a wet dormant season and a relatively wet growing season.

The summary of climate description between 1959 and 1988 for Coimbra appears in Table 10.

Table 10 - Climate description of the vineyard region for 1959-88 (data from Coimbra).

Year	Month	Frost	R. Humidity	Insolation	Ta_{max}	Ta_{min}	T_{max}	T_{min}	Wind speed
1959-88	1	4,6	85,1	122,0	18,6	1,1	14,0	4,4	5,9
	2	2,3	83,8	132,0	20,4	0,9	14,9	5,1	6,2
	3	1,2	78,7	177,5	23,6	1,2	16,9	6,3	6,0
	4	0,7	76,1	200,0	25,6	2,3	18,7	7,6	6,0
	5	0,0	75,4	228,8	29,8	3,9	21,0	9,8	5,7
	6	0,0	74,8	238,8	34,0	7,3	24,7	12,9	5,4
	7	0,0	75,4	278,8	35,5	8,8	27,2	14,2	5,5
	8	0,0	76,3	283,3	35,9	8,3	27,6	13,7	5,3
	9	0,0	78,2	211,6	34,7	7,3	26,4	13,1	4,6
	10	0,0	81,3	181,1	29,0	4,1	22,2	10,5	4,5
	11	2,1	83,9	138,2	23,2	2,3	17,2	7,0	5,7
	12	4,5	84,8	123,3	19,2	0,8	14,3	4,8	6,0

3.3 Experimental design

The trials were performed in three consecutive years, following an experiment designed in a randomized type, with four blocks (replications), and five modalities were applied in each block, using 3 previously marked vines in each modality/block (Table 11):

(1) Control (no intervention) - CTR;

(2) Manual leaf removal at fruit set - removing 6 basal leafs at full bloom - MAD;

(3) Mechanical leaf removal at fruit set - partial defoliation by machine - MED;

(4) Manual Bunch thinning - bunch removal at *veraison*, leaving one per shoot - MBT;

(5) "*Double Maturation Raisonnée*" - "separation" of 85% of the shoots from the plant at least 2 weeks before harvest - DMR.

Two additional modalities were introduced in 2013 vintage in order to have a comparison with the 5 modalities studied:

DMR30 - "separation" 85% of the shoots from the plant at least 30 days before harvest;

BIO - usage of a bio stimulant, *Ascophyllum Nodosum*, before bloom.

Table 11 - Randomized blocks experimental design for all modalities, conducted in experimental vineyard Baga in 2011, 2012 and 2013 vintages.

Block 1	**1 - CTR**	**1 - MAD**	**1 - MED**	**1 - MBT**	**1 - DMR**
Block 2	**2 - MBT**	**2 - DMR**	**2 - MED**	**2 - CTR**	**2 - MAD**
Block 3	**3 - MAD**	**3 - MBT**	**3 - MED**	**3 - DMR**	**3 - CTR**
Block 4	**4 - DMR**	**4 - CTR**	**4 - MED**	**4 - MAD**	**4 - MBT**

Each experimental unit has 14 vines, so it gives a total of 56 vines per modality and a total of 280 vines. For each modality, 12 vines (3 vines per unit) are marked between bud break and flowering to perform the necessary quantifications and a single shoot was marked for the quantifications of leaf area.

3.4 Methods and quantifications

The following methods, techniques and quantifications were implemented with the intention of studying the effects of early leaf removal (manually and mechanically removed), manual bunch thinning and '*double maturation raisonnée*' as yield control techniques. Although primary focused in the viticulture features, this study also covered qualitative aspects regarding fruit quality and wine quality.

3.4.1. Pruning

Pruning was performed on January (10-01-2012, 21-01-2013, and 15-01-2014), of all marked vines of the study; another 3 vines per block and modality were also pruned in order to be used in the study, if needed.

Double Guyot pruning (Figure 7) was performed manually and the pruning wood of each pruned vine was weighted with a standard scale (Korona, Hans Dinslage GmbH, Uttenweiler, Germany) immediately after the task was completed.

3.4.2. Canopy management

All canopy management procedures were carried personally or by 'Caves Messias' (when mentioned in the text) according to the production needs, except for leaf removal, bunch thinning and DMR procedures, where the tasks were performed personally. Early defoliation, bunch thinning and DMR were only performed in the marked vines for those individual study modalities.

3.4.2.1. Suckering

The removal of shoots growing in the old wood, or suckering, was performed manually since the beginning of each vintage. Every task performed in the vineyard was also used to examine abnormal growth of shoots and to remove them promptly.

3.4.2.2. Shoot positioning

Shoot positioning was performed whenever needed, manually and adjusting the movable wires.

3.4.2.3. Shoot trimming

Shoots in cv. Baga usually grow horizontally and vertically (a significant part of the shoots grow horizontally) so shoot trimming is a very important task (complemented with shoot positioning) in order to control the vegetative growth of the plants and to facilitate the usage of machinery. Lateral shoot trimming was performed once or twice between fruit set and *veraison*; shoot trimming of the top of the vine was performed close to *veraison*, in June or July, usually only once and depending on the vegetative growth of the plants - performed to avoid cluster shading due to longer shoots. Shoot trimming was performed by 'Caves Messias' personnel, decided according to vegetative growth of each particular vintage.

3.4.3. Leaf removal

Leaf removals of the vines assigned to modalities MAD and MED were performed between flowering stage (Eichhorn Lorentz E-L stage 23) and fruit set (E-L stage 27). The execution of leaf removal was based on the plant growing season, and the availability of the mechanical leaf removal equipment.

The intensity of the MAD was 6 basal leaves (Figure 8), without removing any lateral leaves. Not removing laterals was considered positive in order to protect the clusters from excessive sunlight during later stages of ripening. MED removed principal and lateral leaves, and occasionally, complete shoots.

MED was performed by leaf removal machinery using an ERO defoliator (ERO GmbH, Niederkumbd, Germany), by passing the machinery twice by each side of the vine row (Figure 9).

Figure 8 - MAD - Manual leaf removal and vines after MAD.

Figure 9 - MED - Mechanical leaf removal and vines after MED.

3.4.4. Manual Bunch Thinning (MBT)

Manual bunch thinning (MBT) was performed at *veraison* (E-L Stage 35), leaving only one cluster per shoot, by removal of all the clusters per shoot except the basal cluster (Figure 10).

If the basal cluster was damaged, or affected by disease, then the basal cluster was removed, and not the upper cluster. Previously, it was chosen to keep the basal cluster, disregarding the ripening stage and/or overall cluster quality when compared with the other clusters, removing the bias of MBT due to choosing the worst cluster as the ones to be removed (and instantly producing better averages).

Figure 10 - MBT – Manual bunch thinning and vines after MBT.

3.4.5. '*Double Maturation Raisonnée*' (DMR)

DMR was performed by cutting each shoot containing clusters immediately below the basal cluster, 15 days before the expected harvest date (Figure 11). The shoots were separated from the vine and left hanging in the upper wire, preferably.

Another modality of DMR was tested in 2013, involving a period of 30 days between the shoot cutting and harvest. The aim of this test was to understand if changing the number of days between DMR and harvest could result in grapes of different quality. This modality is mentioned in text, tables and figures as **DMR30**.

Figure 11 - DMR - *Double maturation raisonnée* and shoots after DMR.

3.4.6 *Ascophyllum nodosum* as bio stimulant.

Ascophyllum nodosum is common brown seaweed (Phaeophyceae) from Fucaceae family, being the only species in the genus *Ascophyllum*, and common on the northern Atlantic Ocean. This seaweed has several uses in agriculture, like nutrient provider and stimulant of vegetative activity. The intended usage of *Ascophyllum* was to study the ability of the seaweed to stimulate rachis growth and to enlarge cluster length, trying to achieve a lower compaction of the cluster. The application of the product was performed only in 2013 vintage, in 22/05/2013, when the plant was in stage E-L 17 (12 leaves separating, and formation of the cluster).

The obtained result was insignificant but the ripening and wine making procedures continued as previously scheduled because the grapes had different quality than all other modalities. This modality is mentioned in text, tables and figures as **BIO**.

3.4.7 Timelines

The timing of performing each modality/activity and the vegetative evolution of the plants during the three vintages is depicted in Table 12.

Table 12 - Dates for each activity and vegetative evolution for 2011 - 2013 vintages.

Eichorn-Lorenz Stages	2011	2012	2013
5 - Bud break	n.a.	26/03/2012	n.a.
9 - Shoots 2-4 cm	n.a.	18/04/2012	n.a.
12 - Shoots 10 cm	04/04/2011	11/05/2012	22/04/2013
15 - 8 leaves separated	n.a.	n.a.	14/05/2013
17 - 12 leaves separated	18/04/2011	n.a.	22/05/2013
19 - Flowering begins	27/04/2011	23/05/2012	n.a.
23 - Full Bloom	04/05/2011	01/06/2012	05/06/2013
25 - 80% caps off	11/05/2011	n.a.	n.a.
27 - Setting	16/05/2011	08/06/2012	14/06/2013
29 - Berries pepper-corn size	20/05/2011	n.a.	20/06/2013
31 - Berries Pea Size	n.a.	22/06/2012	n.a.
33 - Berries hard and green	n.a.	13/07/2012	26/07/2013
35 - Veraison	21/07/2011	08/08/2012	10/08/2013
38 - Harvest	16/09/2011	19/09/2012	23/09/2013
Prunning	10/01/2012	21/01/2013	15/01/2014
MAD	16/05/2011	08/06/2012	05/06/2013
MED	16/05/2011	19/06/2012	06/06/2013
MBT	28/07/2011	13/08/2012	31/07/2013
DMR	26/08/2011	14/09/2012	09/09/2013
DMR 30	n.a.	n.a.	30/08/2013
BIO	n.a.	n.a.	22/05/2013

(n.a. - not available or not applicable).

Harvest dates were chosen according to 'Messias' harvest dates - companies' harvest was performed by machine so the harvest for the study had to be done before the harvester machine crosses the experimental blocks.

In 2011 and 2012, the harvest occurred sooner than expected because of rain incidence. In both years, 'Messias' company had to harvest sooner as well.

3.4.8. Canopy assessment

3.4.8.1. Leaf area estimation

Foliar area of each vine was assessed at full bloom (E-L 23), before and after the defoliation, and at *veraison* (E-L 35) using the methodology described in literature [292], one tagged shoot per vine, 3 vines per repetition.

In order to estimate leaf area (LA), several items were quantified:

Principal leaf number of the shoot; Length of the left and right leaf blades of the larger principal leaf; Length of the left and right leaf blades of the smaller principal leaf; Lateral leaf number of the shoot; Length of the left and right leaf blades of the larger lateral leaf; Length of the left and right leaf blades of the larger lateral leaf.

After performing these quantifications, principal, lateral and total leaf areas were calculated for each vine.

3.4.8.2. Point Quadrat Analyis

Canopy characterization was performed according to Point Quadrat Analysis (PQA) [183]. PQA is a simple method for measuring important parameters of canopy architecture, quantifying canopy differences and providing some insight into vine performance.

Figure 12 - PQA measurement spots.

Measurements were done at constant heights (at lower wire height, close to the fruiting zone, and at upper wire height), by inserting a long rod in specific points of the canopy: 6 insertions were performed, 3 at lower wire height (centre of the vine, 40 cm left from the centre, and 40 cm right from the centre), and 3 at upper wire height (centre of the vine, 40 cm left from the centre, and 40 cm right from the centre).

As the rod contacts any part of the plant, the contacts are identified and recorded - 'leaves' and/or 'clusters' - or 'gaps' if there was no contact with the plant.

From PQA measurements, it can be calculated parameters associated with plant vigour (canopy gap percentage and leaf layer number) and plant light environment (interior leaves percentage and interior clusters percentage). These parameters can then be used to compare vine canopy and also establish if a particular technique to be studied is effective or not.

3.4.9. Yield components

3.4.9.1. Fertility

In order to estimate fertility and the influence of each modality, several parameters were quantified per vine, for each marked shoot between E-L Stages 17 and 19 (separate clusters): number of buds, number of shoots, number of clusters per vine, and the number of flowers per cluster.

The data recorded for the 2011 vintage was used as the initial state of the vineyard and used as contrast with the parameters measured in 2012 and 2013.

Bud break Index = (Shoot number/Bud number) x 100

Potential Fertility Index = (Cluster number/Shoot number)

3.4.9.2. Fruit set

A method described in literature [228] was used to quantify the fruit set ratio for each vine, and modality. Fruit set percentage was determined using the flower number quantified at bloom (determined by a photograph of the cluster at bloom against an orange background - complementary colour of the green cluster) and the number of berries counted at harvest, according to the formula:

$$\text{Fruit set} = (\text{Berry number}/\text{Flower number}) \times 100$$

To achieve this, 20 unmarked basal cluster per vintage were photographed against an orange sheet (complementary colour of cluster green colour); the cluster was then used to count the number of flowers. Afterwards, and using the photographs, the number of visible flowers was counted, and used to calculate a linear regression between visible flowers in the photograph and the actual number of flowers (Figure 13).

Using the visible flower number and the linear regression determined with the unmarked clusters, the estimate number of the flowers per cluster could be determined.

Fruit set was calculated using the flower number quantified at bloom and the number of berries counted at harvest.

Figure 13 - Basal cluster photograph for flower number determination.

3.4.9.3. Cluster components

The marked cluster from each vine were picked separately at harvest (weighted by a standard scale immediately after picking), being subsequently used for quantification of cluster components:

- weight of marked cluster: weighted using electronic scale (Korona, Hans Dinslage GmbH, Uttenweiler, Germany);
- number of berries of each basal cluster: counted after destemming the cluster manually;
- berry weight: calculated by dividing the cluster weight with the number of berries of the respective cluster.

3.4.9.4. Cluster compactness

Cluster compactness was determined at harvest using OIV 204 standard [293], by examination of the marked clusters, in a 5-point scale ranging from (1) very loose to (9) very dense.

3.4.9.5. Botrytis impact

The incidence of rot (*Botrytis cinerea)* was assessed at harvest by visually estimating the fraction of berries with visual symptoms to total berries in each tagged bunch [228].

3.4.9.6. Berry components

Berry weight was measured using 200 berries and a standard scale.

Berry number per bunch was determined by manually separating the berries from the bunch and hand counted.

Berry variability [228] was evaluated using 30 berries taken from several marked clusters of each modality, and weighed using a standard scale. The diameter of each sample berries was measured, and berries were then sliced in half, the seeds and flesh carefully removed from each berry half, without rupturing pigmented hypodermal cells, and the seeds was carefully separated from the flesh. Both skins and seeds were then weighed. All these measurements were performed within 24 hours after harvest.

3.4.9.7. Ripening assessment

Fruit ripening was monitored between *veraison* (E-L Stage 35) and the harvest (Figure 14). The progress of ripening was followed performing determinations using 200 berries samples (for each modality, at each ripening date) of the following parameters: Brix degree and must density, pH and titratable acidity.

Must acidity was determined according to Method OIV-MA-AS313-01 [294].

pH of must was determined according to Method OIV-MA-AS313-15 [295].

Brixº must was determined according to Method OIV-MA-AS2-02 [296].

Must density was determined according to Method OIV-MA-AS2-01B [297].

Sugar load levels per berry were calculated from sugar concentration and berry volume [298,299,300]. It is understood that sugar load can be used as a measure of ripening progression: as it represents the amount of sugar present inside the berry, ripening progression should be considered the increasing progression of the amount of sugar present in each berry.

Figure 14 - Ripening assessment.

3.4.9.8. Harvest

At harvest, the number of clusters and their overall weight per marked vine were recorded immediately using a standard scale.

The average cluster weight of each marked vine was calculated afterwards using these two parameters.

3.4.10. Micro-fermentations

Marked clusters were picked individually and kept separately, the rest of the clusters were picked for separate plastic bags - cluster number and total yield were recorded immediately after finished picking. When arrived at the winery, all bags were split by modality. The clusters were taken off the bags and hand-destemmed into a large thick plastic bag, one bag for each modality - rotten berries were separated and were discarded. After destemming all clusters, the berries were crushed by hand and feet; then placed into 45 litres plastic bins, where the alcoholic fermentation occurred - 30 ppm of sulphur dioxide was added to each bin, to prevent enzymatic oxidation and/or disease spreading. Every must was inoculated the day after, with commercial yeast Viniferm CT007 (AGROVIN, Spain) - this commercial yeast was chosen because its characteristics (ensures polyphenolic and colour stability, while produces low quantity

of fermentation aroma compounds preserving varietal aroma) and to certify that all fermentations, within the vintage and between vintages, occurred with similar yeast population.

Alcoholic fermentation occurred in a stable room temperature, for the time needed, and performing two cap movements per day (punch-downs).

After finishing alcoholic fermentation, the wine was racked from the skins and pomace into small plastic bins, to perform the malolactic fermentation. The bins were filled with wine to assure almost complete absence of air and oxygen.

After the end of malolactic fermentation, the wines were racked for individual plastic bins, added 90 ppm of sulphur dioxide and bottled (glass bottles and closed with corks).

3.5 Chemical analysis of wines produced

Wine chemical characterization was performed at a private laboratory operating according to OIV, IVV and '*Instituto dos Vinhos do Porto e Douro*' (IVDP) reference methods. The parameters performed for wine analysis were alcohol content [301], residual sugar content [302] (OIV-MA-AS311-01A), titratable acidity [294] (OIV-MA-AS313-01), pH [295] (OIV-MA-AS313-15), wine colour and total phenolics parameters [303] (OIV-MA-AS2-07B), Hydrochloric acid index [304].

Sugar load levels per berry were calculated from sugar concentration and berry volume. Sugar load can be a measure of ripening progression as it represents the amount of sugar present inside the berry.

3.6 Carotenoids quantification in berry skins

Grape skin carotenoids quantification ("*Precursores aromáticos en Uvas. Análisis de hollejo de uva*") was performed according to method described in literature [305,306], at University of Rioja, Departamento de Agricultura y Alimentación de la Universidad de La Rioja (Logroño, Spain). The substances quantified by the method were: antheraxanthin, β-carotene, chlorophyll a, chlorophyll b, lutein, neoxanthin, pheophytin, violaxanthin and zeaxanthin. Commercial standards of chlorophyll a and b (Fluka,

Buchs, Switzerland) and carotenoids (CaroteNature, Ostermundigen, Switzerland) were used to build calibration curves for quantification.

Grape samples were conserved below -34º C in absence of light until quantification. Transportation was made in similar conditions.

3.7 Aromatic description of wines produced

Wine aroma compounds quantification was performed according to method described in literature [307] at Laboratório de Toxicologia, Departamento de Ciências Biológicas da Faculdade de Farmácia, Universidade do Porto (Porto, Portugal). The substances quantified were: ethyl butanoate, ethyl hexanoate, ethyl heptanoate, ethyl octanoate, ethyl decanoate, ethyl dodecanoate, ethyl 2-methyl-butanoate, ethyl 3-methyl-butanoate, ethyl *trans*-4-decenoate, *iso*amyl acetate, phenylethyl acetate, diethyl succinate, isoamyl hexanoate, phenylethyl alcohol, benzyl alcohol, α-terpineol, β-*cis*-terpineol, β-linalol, nerolidol, *cis*-β-farnesene, *trans*-α-bisabolene, *trans*-nerolidol, 4-ethylguaiacol, di-hydropseudo-ionone, β-damascenone, limonene, terpinolene, α-pinene, geranyl-acetone, ethyl-2-hexenoate, unidentified sesquiterpene 1, unidentified sesquiterpene 2, unidentified sesquiterpene 3, unidentified sesquiterpene 4, unidentified terpene 1, unidentifid terpene 2, TDN, neryl acetate, ethyl malate, 4-ethyl phenol.

The volatile compounds studied were (CAS number in brackets): limonene (5989-54-8, Fluka), cis-linalool oxide (5989-33-3, Fluka), terpinolene (586-62-9, Aldrich), β-linalool (78-70-6, Sigma), β-terpineol (138-87-4, Sigma), α-terpineol (98-55-5, Sigma), nerol (106-25-2, Aldrich), geraniol (106-24-1, Sigma), α-ionone (6901-97-9, Aldrich), nerylacetate (141-12-8, Aldrich), β-ionone (6901-97-9, Aldrich), nerolidol (7212-44-4, Aldrich), ethyl butanoate (105-54-4, Merck), *iso*amylacetate (123-92-21, Sigma), ethylhexanoate (123-66, Sigma), hexylacetate (142-92-7, Merck), diethylsuccinate (123-25-1, Merck), ethyloctanoate (106-32-2, Merck), phenylethylacetate (103-45-7, Merck) and phenylethyl alcohol (60-12-8, Sigma). A hydrocarbon mixture C_6-C_{20} was obtained from Fluka. NaCl and NaOH were purchased from Merck.

3.7.1 Short chemical and sensorial description of quantified compounds

Annex I has a small description about each quantified compound, regarding nomenclature and odour description, based on an online library, The Good Scents Company [308].

Table 13 shows the odour threshold limits for each quantified aromatic compound, odour descriptor and odorant series. Additional information can be consulted in annex I.

Table 13 - Compounds quantified by SPME-GC-MS method, odour descriptors, odorant series and odour threshold.

Compound	Odour threshold (µg/L)	Odour descriptors	Odorant series
ethyl butanoate (mg/l)	**20**	Papaya, pineapple, fruity, juicy, fruit.	1
ethyl hexanoate (mg/l)	**14**	Apple peel, pineapple, fruity, waxy, estery, green banana	1, 3, 4, 7
ethyl heptanoate (mg/l)	**22**	Fruity, pineapple, sweet, estery, banana, strawberry, cognac, green, spicy, oily	1, 3, 4, 5, 7
ethyl octanoate (mg/l)	**50 - 600**	Fruity, sweet, pear, pineapple, banana, apricot, fat, waxy, musty, wine, mushroom	1, 2, 3, 4, 6, 7
ethyl decanoate (mg/l)	**23 - 200**	Grape, apple, dry fruit, solvent, oily	1, 7
ethyl dodecanoate (mg/l)	**400 - 1500**	Sweet, waxy, soapy, rum, cream, floral	1, 2, 6, 7
ethyl 2-methyl-butanoate (A/10^7)	**18**	Apple, fruity, fruity, fresh, berry, grape, pineapple, mango, cherry	1, 3
ethyl 3-methyl-butanoate (A/10^7)	**3**	Apple, fruity, pineapple, green, orange, sipce	1, 3, 5
ethyl *trans*-4-decenoate (A/10^7)	not available	Green, fruity, oily, pineapple, apple, waxy	1, 3, 7
***iso* amyl acetate (mg/l)**	**30**	Banana, estery, apple	1, 3, 7
phenylethyl acetate (mg/l)	**250**	Rose, honey, Sweet, floral, yeasty, honey,cocoa, balsamic	2, 3, 4, 7
diethyl succianate (A/10^7)	**200000**	Cheese, earthy, spicy, cooked apple, ylang	2, 5, 6, 7
***iso* amyl hexanoate (A/10^7)**	**30**	Fruity, sweet, pineapple, pungent, sour cheese	1, 4, 6, 7
phenylethyl alcohol (mg/l)	**10000 - 14000**	Rose, lilac, bread, honey	2, 4, 7
benzyl alcohol (A/10^7)	**200000**	Flowery, sweet, rose, phenolic, balsamic	2, 3, 4, 7
α-terpineol (A/10^7)	**400**	Floral, citrus, sweet, pine, lilac, woody	1, 2, 3, 7
β-*cis*-terpineol (A/10^7)	**400**	Pungent, earthy, woody	3, 7
β - linalol (mg/l)	**25**	Muscat, citrus, fresh, floral, lavender, sweet, waxy	1, 2, 3, 4, 7
nerolidol (A/10^7)	**700 - 2250 - 10000**	Floral, green, citrus, woody, waxy	1, 2, 3, 7
limonene (µg/l)	**200**	Lemon, orange, citrus	1
terpinolene (A/10^7)	**0.2**	Citrus, Fresh, Pine, Plastic, Sweet, Woody	1, 3, 4, 7
s-α-pinene (A/10^7)	**0.006**	Pine, resin, turpentine	3, 7
geranyl acetone (µg/l)	**186**	Earth, Fatty, Floral, Fresh, Fruit, Green, Herbaceous, Magnolia, Meat, Nut, Rose, Spicy, Tropical, Wax, Wine	1, 2, 3, 5, 6, 7
neryl Acetate (µg/l)	**880 - 905400**	Floral, Rose, Fruity, Raspberry	1, 2
***cis*-β-farnesene (A/10^7)**	not available	Green apple, gardenia, green, citrus, woody	1, 2, 3, 7
***trans*-α-bisabolene (A/10^7)**	not available	Balsamic, oregano, citrus	1, 5, 7
***trans*-nerolidol (A/10^7)**	**700**	Green, floral, woody, fruity, citrus, melon	1, 2, 3, 7
4-ethylguaiacol (A/10^7)	**0.05 - 33**	Spicy, clove-like medicinal, woody, sweet vanilla, animal, barnyard, stable, phenolic, mousy	2, 5, 7
4-ethylphenol (A/10^7)	**440**	Smoke, savory, animal, barnyard, stable, phenolic, and mousy	2, 5, 7
dihydro pseudo-ionone (A/10^7)	not available	Sweet, waxy, citrus, floral, balsamic, dry, dusty, powdery, spicy	1, 2, 4, 5, 7
β-damascenone (µg/l)	**0.05**	Natural sweet, fruity, rose, plum, grape, raspberry, apple, honey, sugar, smoky, tobacco	1, 2, 4, 7
TDN (A/10^7)	**2**	Licorice, petrol	5, 7
ethyl-2-hexenoate (A/10^7)	**0.001**	Sweet, fruity, juicy, rum, green, vegetal	1, 3, 4, 7
ethyl malate (A/10^7)	not available	Caramel, sugar, brown sugar, sweet, wine, fruity, herbal	1, 3, 4, 7
unidentifid terpene 1 (A/10^7)	not available	non available	non available
unidentifid terpene 2 (A/10^7)	not available	non available	non available
unidentified sesquiterpene 1 (A/10^7)	not available	non available	non available
unidentified sesquiterpene 2 (A/10^7)	not available	non available	non available
unidentified sesquiterpene 3 (A/10^7)	not available	non available	non available
unidentified sesquiterpene 4 (A/10^7)	not available	non available	non available

1 = Fruity; 2 = Floral; 3 = Green, Fresh; 4 = Sweet; 5 = Spicy; 6 = Fatty; 7 = Others

3.8 Sensorial analysis of wines produced

Sensorial analysis of the produced wines and wines named Baga references was performed by an expert panel, composed by 7 tasters (three females, four males, ages between 28 and 62 years) with large experience in wine industry and wine tasting (experience between 5 and 44 years).

The descriptive analysis was performed in 2014, after all fermentations ended, and all the wine samples at once. Tasting glasses were ISO grade. Instructions were given to avoid scoring depending on the vintage/age of the wine. A first stage was implemented for training the panel and for normalizing the sensorial definitions and intensities of the different aromas - several commercial aromatic essences were used for training.

The tasters had to score using a 0 to 5 scale (from 0 meaning 'non-existent'; 1 meaning 'slight notes'; 2 meaning 'perceptive'; 3 meaning 'perceptive significantly'; 4 meaning 'perceptive with average intensity'; until 5 meaning 'intense') a list of previously selected aroma descriptors; the tastings were limited to aroma analysis, and the list of previously selected aromas was selected using the aromas reported as varietals for Baga variety wines (see annex III)

The sensorial analysis results were subsequently evaluated by statistical analysis.

3.9 Statistical Analysis

Results of comparison of mean values, using the SPSS v.23 program (IBM SPSS program, version 23, 2015), are expressed as the level of significance. Where resulting differences were significant, individual means were compared using the Duncan's multiple range test ($p < 0.05$), identifying different levels of significance with different letters.

The information is not completely translated by a single variable and therefore univariate data contains marginal information rather than complete information. We might be able to obtain a 'data structure' from observations and also 'noise', that can arise from other components, from the measurement, from the instrument, etc. The separation of the important data structure related to our study and the "noise" (information that has no significance as it is due to variations of instrumental signal and other processes) is a major problem when we are analyzing a set of observations, even

because the data structure hides part of the information when we analyze only one variable. Principal Component Analysis (PCA) is one of the most basic and useful multivariate data analysis tool [309,310,311]. PCA involves the decomposition of a data matrix into 'structure' and 'noise', that is, variance components. The concept of variance is very important - the fundamental assumption of multivariate data analysis is that the maximum variance is associated with the 'hidden phenomena'. What is wanted with this type of data analysis is to determine the "phenomena" that are not visible in the matrix (which is extremely complex) of the data and in the graphical representations of this. It is assumed in PCA that the directions of maximum variance are somehow directed toward these "phenomena". In PCA, the Cartesian axes of the graphic representations of the data matrix are replaced by new axes. These new Cartesian axes are the directions with the maximum variance. The axis PC1, i.e., the first Principal Component axis (PC) is the direction of the maximum longitudinal variance of the data. Another way of looking at the PC1 axis is as the line resulting from the application of the least squares method to the set of points in the matrix. Thus, there are two ways to get the main axis: find the direction with the maximum of longitudinal variance or the direction with the least squares of the distances to the axis itself (transverse distances).

The most frequent choice for the centre of the axes is the midpoint of the entire data matrix. After determining the first PC axis, the remainder are determined from the largest longitudinal variances, decreasingly, and always orthogonal to each other. It is then determined a new set of Cartesian axes. The maximum number of axes is the smallest value of (n-1) objects or p variables. However, not all of the given PCs are normally used. Knowing how many axes to use is a key issue in PCA.

Each PC can be represented as the linear combination of p vectors of the variables, where the coefficients for each variable relative to a given PC are called "Loadings". The loadings give information about the relationship between the original variables and the PCs. The distances of each object to PCs are called "Scores". They are therefore the coordinates of each object relative to the PC axes. The residual values are the projection distances of each object and represent the amount of information lost due to the approximation made by the PCA.

The purpose of PCA is to highlight the relationship between data (observations or individuals) and variables. The process for this is to replace the set of Cartesian axes with new main component axes, reducing (if possible) the number of dimensions of the data matrix, eliminating noise (not important information).

Principal component analysis was performed using Unscrambler v.9.7 program, (CAMO Software, version 9.7, 2007).

IV Viticulture studies - Results and discussion

4.1 Introduction

The Baga is an *ex-libris* red variety in Bairrada and it is required to compose the blend in order to have a wine labelled as '*Bairrada Clássico'* - “Classic Bairrada”. Nevertheless, for Bairrada region the importance attributed to this variety has as many ‘haters’ than ‘lovers’. Some authors mentioned that this variety is not able to produce wines of great ‘finesse’; however in Dão region (adjacent wine producing DOP region of Bairrada) producers improved to higher quality when the percentage of planted Baga variety decreased [181]; the wines are described of harsh dry tannins and forest fruits when young age; when ripe (considered when having 13% alcohol or above) and after some ageing time (in barrels or/and in bottle), develop depth and complexity, the tannins soften, and the aroma got nuances of black plums, herbs, olives, smoke, tobacco, honey, camphor, and coffee [312].

Briefly, Baga variety can be described as having compact clusters and thin skin berries, two characteristics that can lead to rupture of the berries and appearance of rot, especially if precipitation occurs near maturity/harvest date. Baga vines often develop with excessive vigour and high yield, and almost always in need cluster/bunch thinning, as is frequently mentioned by Baga producers [6]. Bunch thinning is usually performed four weeks before the expected harvest date. If it occurs later, the thinned clusters can be used for the production of sparkling and rosés wines, avoiding the fall in yield of the plot. It is believed that it produces its best wines in calcareous-clay soils.

According to some producers [6], cluster thinning helps to achieve the later stages and perfect maturation of Baga, improving also the phenolic ripeness. Some of them mention that is almost impossible to achieve great quality in Baga variety without performing bunch thinning. Additionally, the ungrafted vines tend to produce less but with better quality, also with better phenolic concentration (ungrafted vines tend to be older, before mass clonal selection, and also using different trellising).

4.2 Weather characterization

As it can be seen from the data (Table 9), the three years of the study had different weather: 2011 was a warmer year (Winkler's 'region IV' and Huglin's 'Warm' classifications), and rained less during the growing season (low Selianinov index value), although having a good volume of rain during dormant season; 2012 was cooler year (Winkler's 'region III' and Huglin's 'Warm/Temperate' classifications), with a dry dormant season but a wet growing season (very high Selianinov index value); 2013 was also a cool year (Winkler's 'region III' and Huglin's 'Warm/Temperate' classifications), with a wet dormant season and a relatively wet growing season (high Selianinov index value).

Table 14 - Climate description, using several bioclimatic indexes, of the vineyard region for 2011 - 2013 vintages.

Climate description	2011	2012	2013
Daily Av. Temperature Max. (º C)	22.3	21.5	20.9
Daily Av. Temperature Min. (º C)	11.5	10.0	10.7
Daily Av. Temperature (º C)	16.3	15.1	15.4
Daily Av. Sunshine (h)	7.4	7.3	7.0
Yearly Sunshine (h)	2691	2647	2562
Daily Av. Precipitation (mm)	2.0	1.8	2.5
Yearly Precipitation (mm)	719	639	921
Nº days Tmin<10º C Oct-Feb	94	102	99
Nº days Tmin<7º C Oct-Feb	74	91	71
Nº days Tmin<10º C Mar-Sep	28	51	54
Precipitation 4 weeks around flowering (mm)	75.0	23.4	62.4
Nº days raining 4 weeks around flowering	12	15	14
Av. Temp 4 weeks around flowering (º C)	20.3	18.6	16.4
Nº days <10º C 4 weeks around flowering	3	0	5
Av. Temp 2 weeks before fruit set (º C)	19.3	19.0	17.3
Av. Min. Temp 2 weeks before fruit set (º C)	12.7	14.7	12.4
Av. Temp 2 weeks after fruit set (º C)	21.4	18.2	20.0
Av. Min. Temp 2 weeks after fruit set (º C)	15.8	13.4	14.1
Nº days >30º C Mar-Sep	37	31	35
Nº days >30º C Aug-Sep	8	18	21

Bioclimatic indexes	2011	2012	2013
Winkler Index (º C)	2220	1911	1915
Huglin Index (º C)	2453	2184	2226
Cool Night Index (º C)	16.8	15.2	14.3
Branas Heliothermic Index (º C h)	4.0	3.0	3.4
Hydrothermic Index (º C mm)	3109	3478	2136
Selianinov Index (mm/º C)	2.3	8.4	6.5
Σ Growing season Temperature (º C)	2089	1773	1815
Growing season precipitation (mm)	188	288	219
Growing season average Temperature (º C)	19.9	18.6	18.6
Dormant season Precipitation (mm)	513	273	570
Σ Dormant season Temperature (ºC)	1669	1648	1662
Σ Dormant Daily Av. (High - Low) T (º C)	1511	1772	1240
Σ Aug-Sept Daily Av. (High - Low) T (º C)	593	814	880

Some interesting features summarized in Table 14, that may have had importance for the overall harvest yield and quality, must be mentioned:

In 2011, there were 28 days in the growing season when the minimum temperatures fallen below 10º C, opposing with 51 days in 2012 and 54 days in 2013;

In 2012, there were 91 days in the dormant season when minimum temperatures fallen below 7º C (102 days below 10º C), contrasting with 74 days in 2011 and 71 days in 2013;

Precipitation around flowering in 2012 was 23.4 mm, differing from 62.4 mm in 2013 and 75.0 mm in 2011;

The average temperature around flowering in 2013 was 16.4º C, different from 18.6º C in 2012 and 20.3º C in 2011;

The number of days that minimum temperatures around flowering fallen below 10º C in 2012 was 0, contrasting with 3 days in 2011 and 5 days in 2013;

Finally, the number of days that maximum temperatures were above 30º C during the ripening period (August and September) in 2011 was 8, divergent from 18 days in 2012 and 21 days in 2013.

Table 15 - Days in difference between vegetative stages and/or modality activities.

Days of difference	2011	2012	2013
Bloom - Fruitset	12	7	9
Fruitset - Veraison	66	61	57
Veraison - Harvest	57	42	44
Bloom - Veraison	78	68	66
Bloom - Harvest	135	110	110
Bloom - MAD	12	7	0
MAD - Fruitset	0	0	-9
MAD - Harvest	123	103	110
Bloom - MED	12	18	1
MED - Fruitset	0	11	-8
MED - Harvest	123	92	109
Veraison - MBT	7	5	-10
MBT - Harvest	50	37	54
Veraison - DMR	36	37	30
DMR - Harvest	21	5	14
Veraison - DMR30	n.a.	n.a.	20
DMR30 - Harvest	n.a.	n.a.	24
BIO - Bloom	n.a.	n.a.	14
BIO - Veraison	n.a.	n.a.	80
BIO - Harvest	n.a.	n.a.	124

(n.a. - not available or not applicable)

Differences in days between important vegetative stages and/or modality activities were calculated and are presented in Table 15.

Some interesting information can be observed in the timelines of modalities application and vegetative growth stages:

2011 was the year with longest 'bloom to harvest' period, 135 days, and 2012 and 2013 had 110 days - all the periods between stages were longer than in previous years;

MAD and MED occurred at fruit set in 2011 and 2012, but was applied before fruit set in 2013; MED was delayed for several days in 2012 due to a mechanical malfunction of the equipment;

MBT was performed one week after *veraison* in 2011 and 2012, but in 2013 it was applied just before *veraison*;

DMR was performed at least 2 weeks before harvest in 2011 and 2013, but in 2012 was only 5 days before harvest because of unpredicted early harvest (due to rain).

4.3 Canopy characterization and early leaf removal

Canopy description was performed at *veraison* using canopy height and width measurements and Point Quadrat Analysis (PQA) [183]. PQA can be used to evaluate and compare plant vigour expression and also do a characterization of the light and temperature environment close to the fructification zone. Performing PQA at *veraison* helps to understand the form of the canopy during ripening and maturity stages.

Table 16 - Canopy description for 2011, 2012 and 2013 vintages and exposed canopy surface - height and width, determined using 40 vines per modality. Average values and standard deviation (between brackets).

	Modality	2011 Averages	2012 Averages	2013 Averages	3-Vintage Average
Canopy Height (cm)	CTR	98.0 (10.7) a	103.0 (10.7) b	90.9 (13.6) b	97.3
	MAD	97.9 (11.3) a	84.6 (11.3) a	76.2 (12.5) a	86.2
	MED	103.8 (12.7) a	94.1 (12.7) ab	96.3 (10.7) b	98.1
	MBT	101.1 (11.8) a	100.0 (11.8) b	97.6 (8.2) b	99.6
	DMR	99.3 (14.8) a	92.8 (14.8) ab	94.4 (10.8) b	95.5
	All Modality Average	100.0	94.9	91.1	n.a.
Canopy Bottom Width (cm)	CTR	56.8 (7.7) a	54.2 (7.7) a	53.5 (5.8) a	54.8
	MAD	52.3 (9.4) a	53.4 (9.4) a	50.9 (5.1) a	52.2
	MED	54.3 (6.7) a	49.2 (6.7) a	47.3 (6.7) a	50.3
	MBT	55.5 (8.4) a	55.0 (8.4) a	52.8 (8.6) a	54.4
	DMR	52.5 (5.6) a	52.8 (5.6) a	50.3 (6.4) a	51.9
	All Modality Average	54.3	52.9	51.0	n.a.
Canopy Top Width (cm)	CTR	55.8 (7.7) ab	53.8 (7.7) a	43.8 (4.9) a	51.1
	MAD	60.8 (8.9) b	53.7 (8.9) a	48.8 (5.9) ab	54.4
	MED	51.3 (9.4) a	49.2 (9.4) a	51.1 (5.3) b	50.5
	MBT	52.5 (7.9) a	49.3 (7.9) a	45.3 (5.6) a	49.0
	DMR	56.3 (6.0) ab	54.3 (6.0) a	52.9 (5.1) b	54.5
	All Modality Average	55.3	52.1	48.4	n.a.
Calculated Exposed Canopy Surface (cm^2)	CTR	30017	31312	27147	29492
	MAD	30011	25680	22849	26180
	MED	31431	28155	28813	29466
	MBT	30734	30215	29187	30046
	DMR	30227	28169	28471	28956
	All Modality Average	30484.1	28706.3	27293.4	n.a.

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*'). Different letters indicate significantly different averages, according to Duncan test $p<0.05$. n.a. - not available or not applicable.

Vine canopy was similar for all modalities during 2011 and 2012 vintages; for 2013, several differences occurred, the average of each modality were lower than the previous years - vegetative growth was weaker in 2013, and it is reflected in vine height and width being lower (Table 16). Canopy height for MAD lowered gradually from 2011 until 2013, and also lowered for MBT in lesser extent, but not with the remaining

modalities. Although vigour was smaller in 2013, canopy width was higher than the optimal values mentioned in the literature [313] - canopy width between 30 to 40 cm.

Manual and mechanical defoliation reduced the amount of main leaves, main leaf area and total leaf area as compared to control, and also triggered lateral regrowth (Table 17). MAD removed more than 40% of total leaf area in every vintage (46.3% in 2011; 47.7% in 2012; 56.6% in 2013), and MED removed more than 20% of total leaf area (22.3% in 2011; 24.2% in 2012; 30.0% in 2013). Though a large leaf area was removed with MAD and MED, leaf area was compensated until reaching *veraison*, when it was similar from the value recorded in the control vines.

Table 17 - Modality influence over leaf area, for 2011, 2012 and 2013 vintages, determined using 12 marked vines and shoots per modality. Average values and standard deviation (between brackets).

	Modality	2011 Averages	2012 Averages	2013 Averages	3-Vintage Average
Principal Leaf Number/vine (at fruit set)	**CTR**	13.5 (2.5) a	15.8 (1.7) a	11.7 (1.2) a	13.7
	MAD	13.3 (2.6) a	14.7 (1.3) a	11.1 (1.1) a	13.0
	MED	14.0 (2.0) a	15.2 (2.3) a	11.6 (1.3) a	13.6
	MBT	12.2 (2.0) a	15.4 (2.2) a	11.3 (1.6) a	13.0
	DMR	12.7 (1.7) a	15.1 (1.4) a	10.5 (2.7) a	12.8
	All Modality Average	13.1	15.2	11.2	n.a.
Total Leaf Area after defoliation/vine (at fruit set) (cm^2)	**CTR**	17777 (6501) c	21650 (9791) b	17081 (6733) c	18836
	MAD	8901 (3877) a	9018 (3008) a	5880 (3011) a	7933
	MED	12910 (5006) ab	16147 (5710) b	10050 (3477) ab	13035
	MBT	15344 (5849) bc	21824 (6937) b	13540 (6243) bc	16903
	DMR	15066 (3089) bc	18909 (4544) b	12000 (5690) b	15325
	All Modality Average	14000	17509	11710	n.a.
Total Leaf Area/vine (at veraison) (cm^2)	**CTR**	25627 (11523) a	23733 (11741) ab	31707 (11563) b	27023
	MAD	20378 (11816) a	16430 (5137) a	17350 (8743) a	18053
	MED	22138 (12693) a	23914 (11454) ab	21008 (6888) a	22354
	MBT	20883 (7580) a	25167 (10911) ab	25302 (12803) ab	23784
	DMR	20977 (6058) a	25684 (8530) b	21905 (9664) a	22856
	All Modality Average	22001	22986	23455	n.a.

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*'). Different letters indicate significantly different averages, according to Duncan test $p<0.05$. n.a. - not available or not applicable.

The leaf removal machinery performed single-pass defoliation for each side of the canopy in 2011 and some concerns about the efficiency of the defoliation lead to make twice-pass defoliation in 2012 and 2013. The increase of passages from the leaf removal machinery did not cause a significant increase of the percentage of leaf area removed from canopy.

Using PQA to evaluate the plant vigour at *veraison* (Table 18), it was clear that there were minimal variations between 2011 and 2012 vintages - Leaf Layer Number (LLN)

was similar and Percentage of Gaps in the canopy (%Gaps) had a few differences. It should be noticed that %Gaps increased regularly for MBT and DMR modalities from 2011 until 2013, which didn't occurred for the other modalities; it should be considered the hypothesis of this being related with consequences from the modalities procedures. Again, the vigour expression is lower for 2013, with higher %Gaps in the canopy and lower LLN than previous years, for all modalities.

Table 18 - Canopy description and plant vigour expression at *veraison*, using Point Quadrat Analysis - 2011, 2012 and 2013 vintages, determined using 12 marked vines and shoots per modality. Average values and standard deviation (between brackets).

	Modality	2011 Averages	2012 Averages	2013 Averages	3-Vintage Average
% Gaps/vine (Point Quadrat)	CTR	9.8 (15.1) a	8.4 (11.3) a	15.3 (13.1) ab	11.17
	MAD	8.5 (8.9) a	5.6 (10.8) a	20.8 (17.5) ab	11.63
	MED	7.0 (11.2) a	7.0 (11.2) a	8.4 (11.3) a	7.47
	MBT	5.6 (14.8) a	14.0 (15.6) a	26.5 (13.0) b	15.37
	DMR	7.1 (8.8) a	12.6 (12.5) a	26.5 (16.5) b	15.40
	All Modality Average	7.60	9.50	19.50	n.a.
Leaf Layer Number (Point Quadrat)	CTR	3.0 (0.7) a	3.2 (1.0) a	2.6 (0.6) a	2.93
	MAD	2.8 (0.9) a	2.9 (0.6) a	2.2 (0.3) a	2.63
	MED	3.5 (1.1) a	3.2 (0.7) a	2.6 (0.7) a	3.10
	MBT	3.4 (1.1) a	2.8 (0.6) a	2.2 (0.4) a	2.80
	DMR	3.2 (0.7) a	2.9 (0.6) a	2.3 (0.8) a	2.80
	All Modality Average	3.20	3.00	2.40	n.a.

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*'). Different letters indicate significantly different averages, according to Duncan test $p<0.05$. n.a. - not available or not applicable.

Literature mentioned that optimum canopy gap percentage was 40% and leaf layer number between 1 and 1.5 [38] and the quantified values were considerably different from the optimal values (these values were described for Australia and New Zealand and so attention is due when comparing them to Portuguese viticulture context [222]). The percentage of gaps in canopy was much lower, and leaf layer number was always higher, which shows that the canopy was denser than the optimal literature values.

Leaf layer number for MAD was lower than all modalities in 2011 (even not significantly) and similar in 2012 and 2013; MED appeared to be higher than other modalities, which was not expected. PQA was performed at *veraison* and MAD was submitted to severe defoliation at fruit set, so it was reasonable to assume that LLN for MAD could be lower than CTR and MBT, and not similar. This could be explained by intense lateral formation because of the intense early defoliation, compensating the removed leaves. From this it should be mentioned that, although LLN was similar for MAD and MBT, canopies were differently structured - MBT leaf distribution consisted in

principal leaves predominantly, MAD had a strong lateral leaves population and no basal principal leaves, and so MAD leaf age was much younger, on average, than MBT leaf age population, which can be important during ripening period (old basal principal leaves can lose their activity during ripening period due to age) [228]. Additionally, shoot trimming performed traditionally in June-July could also be a factor that influenced leaf area.

Table 19 - Canopy description and light environment characterization at *veraison*, using Point Quadrat Analysis - 2011, 2012 and 2013 vintages, determined using 12 marked vines and shoots per modality. Average values and standard deviation (between brackets).

	Modality	2011 Averages	2012 Averages	2013 Averages	3-Vintage Average
% Interior Leaves (Point Quadrat)	CTR	43.8 (9.8) a	47.6 (10.3) a	51.9 (10.3) a	47.77
	MAD	41.8 (11.7) a	43.9 (10.2) a	52.8 (8.9) a	46.17
	MED	48.0 (9.5) a	43.5 (12.2) a	56.8 (9.3) a	49.43
	MBT	44.8 (14.1) a	45.3 (10.7) a	53.3 (11.2) a	47.80
	DMR	45.0 (9.0) a	44.5 (6.1) a	55.8 (11.4) a	48.43
	All Modality Average	44.70	45.00	54.10	n.a.
% Interior Clusters (Point Quadrat)	CTR	68.0 (35.2) ab	86.1 (30.8) a	45.7 (22.0) bc	66.60
	MAD	46.7 (39.1) a	71.5 (32.7) a	12.8 (21.9) a	43.67
	MED	81.7 (28.4) b	82.4 (36.9) a	27.8 (19.9) ab	63.97
	MBT	81.3 (21.9) b	86.8 (20.5) a	52.1 (25.4) c	73.40
	DMR	91.3 (13.8) b	85.6 (30.4) a	62.4 (34.8) c	79.77
	All Modality Average	73.80	82.50	40.20	n.a.

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*'). Different letters indicate significantly different averages, according to Duncan test $p<0.05$. n.a. - not available or not applicable.

Regarding the light environment of the fructification zone of the canopy, there were no significant differences in the percentage of interior leaves (%IL) within the year, and the values between the years were very similar - higher values for 2013 could be the result of lower vegetative expression, with shorter shoots (Table 19). Concerning the percentage of internal clusters (%IC), the values for 2013 were lower than the previous years, showing once more, the impact of low vegetative expression of the vines. The %IC for MAD was always lower comparing to the other modalities, and CTR, MBT and DMR had higher values.

Canopy structure of all modalities in all vintages was too dense compared with literature, where it was mentioned optimum values lower than 20% of interior leaves and 60% of exposed clusters [38] - the values were far from the optimal values found in literature but attention to the fact that the values are for Australia/New Zealand context and not for Portuguese viticulture.

4.4 Yield components

4.4.1 Bud break and Fertility

Bud break and fertility ratios were described using the number of buds, shoots and bunches per vine, and also the bud break and Potential Fertility indexes (Table 20 and Table 21).

Table 20 - Modality influence over bud number, shoot number and bunch number per vine, for 2011, 2012 and 2013 vintages, determined using 12 marked vines and shoots per modality. Average values and standard deviation (between brackets).

	Modality	2011 Averages	2012 Averages	2013 Averages	3-Vintage Average
Bud number/vine	**CTR**	14.6 (2.2) a	14.2 (3.2) a	15.6 (2.8) b	14.80
	MAD	15.7 (2.5) a	13.2 (3.4) a	12.9 (2.6) a	13.93
	MED	15.8 (2.0) a	12.0 (1.3) a	12.6 (2.0) a	13.47
	MBT	15.5 (3.8) a	13.5 (1.9) a	12.5 (3.4) a	13.83
	DMR	15.6 (2.5) a	13.7 (2.9) a	12.1 (2.6) a	13.80
	All Modality Average	15.40	13.30	13.10	n.a.
Shoot number/vine	**CTR**	13.3 (1.9) a	13.8 (3.9) a	13.8 (2.9) b	13.63
	MAD	15.0 (2.4) a	12.7 (4.5) a	11.5 (2.5) a	13.07
	MED	13.4 (2.0) a	12.7 (2.2) a	10.9 (1.4) a	12.33
	MBT	13.6 (2.5) a	13.8 (1.7) a	11.3 (4.0) a	12.90
	DMR	13.5 (1.7) a	13.9 (3.0) a	11.0 (2.8) a	12.80
	All Modality Average	13.80	13.40	11.70	n.a.
Bunch number/vine (at fruit set)	**CTR**	17.8 (2.4) a	27.8 (9.0) c	24.7 (7.2) c	23.43
	MAD	21.5 (6.2) a	23.8 (8.4) ab	15.6 (6.8) b	20.30
	MED	20.5 (3.8) a	26.9 (9.2) bc	21.2 (7.1) c	22.87
	MBT	19.2 (4.4) a	18.4 (6.3) a	12.3 (5.6) a	16.63
	DMR	20.6 (4.9) a	20.2 (8.4) ab	10.9 (7.1) a	17.23
	All Modality Average	19.92	23.42	16.94	n.a.

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*'). Different letters indicate significantly different averages, according to Duncan test $p<0.05$. n.a. - not available or not applicable.

Bud and shoot number are dependent of pruning of the end of the previous vegetative season and so, if pruning was correctly performed and the vines were balanced, there should be little differences in bud and shoot number between vintages. The values for 2013 were lower than the ones for the previous vintages, and this was also observed for the canopy description. In general, there were no significant differences of number of buds and number of shoots between the modalities, but the averages values were lower year by year.

Bunch number at fruit set was uneven between vintages, and spring climate (between flowering and fruit set) could be responsible for this unbalanced behaviour.

Even though, it should be remarked that CTR bunch number are higher than the other modalities, which could mean than there are post-vintage effects for each modality; MBT and DMR showed lower bunch number than the other modalities and both these aspects could directly and indirectly contribute to CTR having higher yield and MBT and DMR having lower yield.

Table 21 - Modality influence over Bud break and Potential fertility indexes, for 2011, 2012 and 2013 vintages, determined using 12 marked vines and shoots per modality. Average values and standard deviation (between brackets).

	Modality	2011 Averages	2012 Averages	2013 Averages	3-Vintage Average
Bud break index (%)	**CTR**	91.3 (8.7) ab	96.6 (10.1) ab	88.5 (4.1) a	92.1
	MAD	96.1 (8.6) b	94.5 (13.2) a	89.1 (6.6) a	93.2
	MED	85.3 (8.1) a	105.2 (11.5) b	87.6 (8.6) a	92.7
	MBT	89.1 (8.3) ab	102.4 (10.1) ab	89.4 (12.0) a	93.6
	DMR	87.4 (7.9) a	102.1 (11.5) ab	90.9 (11.5) a	93.5
	All Modality Average	89.8	100.2	89.1	n.a.
Potential Fertility Index	**CTR**	1.36 (0.25) a	2.18 (0.98) b	1.82 (0.56) b	1.78
	MAD	1.44 (0.38) a	1.91 (0.51) b	1.58 (0.46) b	1.64
	MED	1.54 (0.25) a	2.11 (0.50) b	1.97 (0.46) b	1.87
	MBT	1.43 (0.32) a	1.32 (0.41) a	0.91 (0.44) a	1.22
	DMR	1.53 (0.34) a	1.25 (0.75) a	0.98 (0.63) a	1.25
	All Modality Average	1.46	1.75	1.45	n.a.

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*'). Different letters indicate significantly different averages, according to Duncan test $p<0.05$. n.a. - not available or not applicable.

Regarding bud break and fertility indexes, results were irregular once more. Bud break increased from 2011 to 2012 and decreased to the lowest values, in 2013. Although significant differences appeared in 2011 and 2012, there were no differences in 2013.

Fertility index had a similar behaviour as bud break: increased from 2011 to 2012 and decreased to 2013. One interesting feature was that fertility in MBT and DMR was significantly lower than the other modalities for 2012 and 2013.

4.4.2 Fruit set

Fruit set was one of the important features to be studied - less berries could imply lower yield (if there was no compensation from the plant, producing less but larger berries). Fruit set percentage was determined using the flower number quantified at bloom (determined by a photograph of the cluster at bloom against an orange background - complementary colour of the green cluster) and the number of berries counted at harvest.

Each vine's basal cluster was photographed against an orange sheet and the photograph was then used to determine the visible flower number. Using the visible flower number and the linear regression determined (Figure 15) with the unmarked clusters, the estimate number of the flowers per cluster could be determined. Fruit set was calculated using the flower number quantified at bloom and the number of berries counted at harvest.

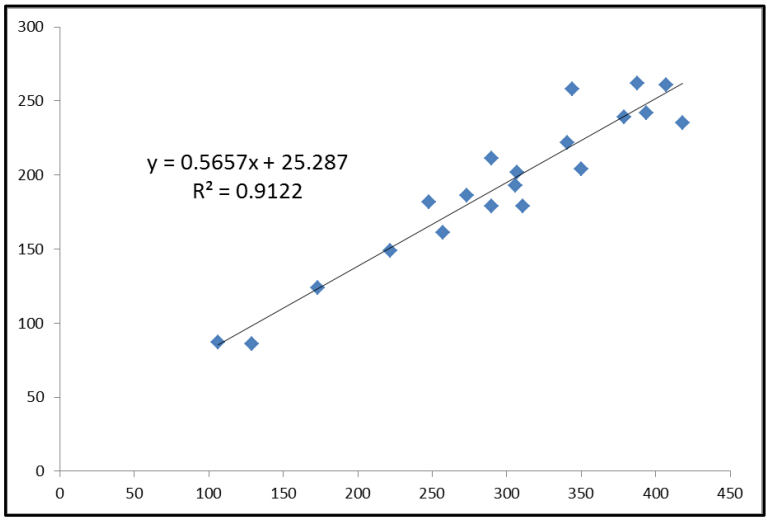

Figure 15 - Linear regression between cluster visible flowers of the photograph and the actual number of flowers, used to estimate marked cluster number of flowers.

Table 22 shows flower and berry number, and respective fruit set ratio for the marked clusters. There were no significant differences in flower number between modalities in 2011 and 2012; there were some differences in 2013, for the modalities that have higher standard deviation. It must be pointed the low number of flowers for MED in 2013, significantly lower than any other modality - this result could be due to the flower destruction from earlier mechanical defoliation.

Table 22 - Modality influence over Flower and Berry number, Fruit set ratio, and berry number ration between CTR and each modality, for 2011, 2012 and 2013 vintages, determined using 12 marked vines and shoots per modality. Average values and standard deviation (between brackets).

	Modality	2011 Averages	2012 Averages	2013 Averages	3-Vintage Average
Flower number	**CTR**	447.4 (129.6) a	428.1 (149.9) a	343.3 (140.7) ab	406.3
	MAD	424.4 (153.5) a	452.9 (147.1) a	363.6 (177.8) b	413.6
	MED	439.7 (95.9) a	441.8 (162.2) a	236.2 (104.4) a	372.6
	MBT	373.9 (127.1) a	405.6 (189.3) a	365.1 (141.5) b	381.5
	DMR	466.3 (188.2) a	438.7 (233.4) a	303.8 (105.5) ab	402.9
	All Modality Average	430.3	433.4	322.4	n.a.
Berry number	**CTR**	136.0 (56.9) b	172.9 (88.9) a	167.5 (37.8) b	158.8
	MAD	75.9 (36.7) a	143.8 (58.6) a	160.0 (65.9) b	126.6
	MED	120.7 (44.0) ab	143.4 (78.2) a	82.4 (64.7) a	115.5
	MBT	99.6 (67.2) ab	121.0 (63.5) a	177.0 (90.8) b	132.5
	DMR	124.9 (40.0) ab	154.7 (64.4) a	134.1 (66.2) a	137.9
	All Modality Average	111.4	147.2	144.2	n.a.
Fruitset (%)	**CTR**	32.2 (10.8) a	43.3 (17.7) a	49.9 (30.0) a	41.8
	MAD	20.0 (15.6) a	34.4 (18.1) a	49.8 (25.1) a	34.7
	MED	30.2 (14.0) a	38.8 (23.2) a	34.1 (29.7) a	34.4
	MBT	27.2 (16.1) a	36.3 (19.9) a	41.7 (32.0) a	35.1
	DMR	30.8 (10.2) a	40.3 (15.9) a	44.8 (36.3) a	38.6
	All Modality Average	28.1	38.6	44.1	n.a.
Berry Number (Modality/CTR)	**CTR**	n.a.	n.a.	n.a.	n.a.
	MAD	0.56	0.83	0.96	0.78
	MED	0.89	0.83	0.49	0.74
	MBT	0.73	0.70	1.06	0.83
	DMR	0.92	0.89	0.80	0.87
	All Modality Average	n.a.	n.a.	n.a.	n.a.

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*'). Different letters indicate significantly different averages, according to Duncan test $p<0.05$. n.a. - not available or not applicable.

Regarding the number of berries, there were significant differences in 2011 and 2013, but they could be more pronounced if the standard deviation of the results were lower. MAD and MED tend to have less berries than CTR, DMR tend to have also lower berry number (because of the berry dehydration, wind could easily separate berries from the clusters), and MBT has lower berry number in 2011 and 2012 but the highest berry number in 2013 (overall, MBT showed to have less berries than CTR). When analysing the berry number ratio of each modality against CTR, the studied modalities had lower berry number than CTR, in the range of 0.7 up to 1.1. The exceptions to this behaviour were MAD in 2011 and MED in 2013, which had lower values, 0.56 and 0.49, respectively. The lower MAD/MED berry number was an already know result [228]. As for MBT and DMR berry numbers, these modalities should not be affected due to their late use but the values are lower than CTR.

Fruit set percentages show no significant differences within the three vintages - this should be related to high variability of the results obtained. Even though there are no significant differences, all modalities reduced fruit set when compared with CTR, vintage average values are different and several features can be pointed:

CTR and DMR have the highest fruit set ratios; MAD has lower fruit set in 2011 and 2012, but equal to CTR in 2013; MED has lower fruit set in 2012 (MED was performed too late in 2012 and might have caused flower destruction) and 2013, but not in 2011; MBT has lower fruit set than CTR. Previous researches have shown low fruit set for MAD and MED [225,228,230,314,315,316] but similar results were not reported for MBT or DMR before. Again, the high standard deviation of the results did not allow significant differences.

One important remark should be noted - average fruit set increases from 2011 to 2013, and these results were due to berry number average in 2013 was similar to 2012, even though being a low vigour year, with lower flower number, with average temperatures lower than 2011 and 2012, and high occurrence of rain in previous days to fruit set.

One might place the question whether the number of berries in a Baga cluster is limited by the length of the rachis - and if the number of viable berries might be similar because of the limiting effect of the rachis length? In 2013 vintage, MED showed lower number of berries but each berry is larger and heavier than berries from other modalities; probably compact clusters do not allow berries to grow and, either berries are compressed and do not complete its format or berries could be expelled from the cluster due to high compression.

4.4.3 Cluster morphology

Cluster morphology (Table 23) was studied using the marked clusters, by determining the cluster weight, average berry weight (calculating from cluster weight and berry number), and cluster compactness.

General trend of results was that CTR clusters had more weight than other modalities, and the berry weight was also higher; DMR had the lightest clusters and berries, and MAD and MED had lighter clusters than MBT. Average cluster weight for 2011 and 2013 was similar, and 2012 had a much higher value. MAD and MED had lighter clusters than CTR and MBT, and DMR clusters were usually the lightest cluster average. So, every modality produced clusters with lower weight than CTR. Several

works mentioned that MAD and MED could produce lighter clusters [314,315,316], and others referred the same for DMR [317,318]. For MBT, the most common result regarding cluster weight show higher weight and not lower [319,320].

In all three vintages, berry weight for every modality was also lighter than for CTR, with the exception of MBT in 2012, probably due to compensation from cluster removal. DMR produced low weight berries due to dehydration. MAD and MED showed lower weight berries when compared with CTR, due to restriction of berry formation [228,314].

Regarding the cluster compactness, CTR had the highest values for the three vintages, and DMR had the lowest values. The 2012 vintages showed homogenous results, probably due to the weather conditions of the year. For all vintages, every modality produced clusters with lower compactness than CTR. MAD [228,314,315] and DMR showed significant lower cluster compactness when compared with CTR as it was expected and MED and MBT showed variable results.

Table 23 - Modality influence over basal cluster morphology - cluster and berry weight, and compactness, for 2011, 2012 and 2013 vintages, determined using 12 marked clusters per modality. Average values and standard deviation (between brackets).

	Modality	2011 Averages	2012 Averages	2013 Averages	3-Vintage Average
Cluster weight (marked cluster) (g)	CTR	261.3 (97.8) b	313.1 (151.7) a	195.0 (55.7) b	256.46
	MAD	110.0 (73.8) a	236.8 (137.5) a	146.5 (46.8) ab	164.44
	MED	178.9 (101.2) ab	205.7 (144.9) a	109.1 (69.0) a	164.56
	MBT	180.4 (119.5) ab	234.0 (169.4) a	185.0 (98.7) b	199.80
	DMR	126.8 (91.8) a	201.6 (85.2) a	87.6 (43.1) a	138.67
	All Modality Average	171.48	238.24	144.64	n.a.
Berry weight (marked cluster) (g)	CTR	1.83 (0.70) c	1.87 (0.61) ab	0.94 (0.50) ab	1.55
	MAD	1.50 (0.75) abc	1.63 (0.43) ab	0.90 (0.38) ab	1.34
	MED	1.20 (0.75) ab	1.33 (0.59) a	1.22 (0.89) b	1.25
	MBT	1.70 (0.58) bc	1.90 (0.73) b	0.75 (0.49) ab	1.45
	DMR	0.98 (0.68) a	1.36 (0.62) a	0.55 (0.42) a	0.96
	All Modality Average	1.44	1.62	0.87	n.a.
Compactness (marked cluster)	CTR	6.8 (0.4) b	6.1 (1.7) a	7.5 (0.8) c	6.79
	MAD	5.2 (2.0) a	5.8 (1.3) a	5.9 (1.4) bc	5.64
	MED	6.4 (1.5) ab	5.8 (1.9) a	5.6 (2.8) b	5.94
	MBT	6.2 (0.8) ab	5.9 (1.6) a	6.9 (1.0) bc	6.34
	DMR	5.5 (2.0) ab	4.5 (0.8) a	3.9 (1.7) a	4.63
	All Modality Average	6.00	5.60	6.00	n.a.

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*'). Different letters indicate significantly different averages, according to Duncan test $p<0.05$. n.a. - not available or not applicable.

4.4.4 Berry components

Berry components were studied using the berry the following parameters: weight average of pulp, skin and seeds, skin/pulp ratio, seed number and berry diameter (Table 24) of 30 berries. Berry weight determination was a different procedure than the average berry determination using the marked cluster - berry weight from marked cluster determination is the average berry weight calculated from the cluster weight and the number of berries of the cluster; berry weight determination is the average weight of 30 berry samples taken at ripening assessment, from several clusters of several vines.

There were small differences between modalities berry weight with the exception of DMR, which had the lightest berries. MAD produced berries lighter than CTR, and MED and MBT showed a fluctuant behaviour over the vintages. According to modality to CTR ration shown in Table 25, the relationship between modalities berry weight with CTR was between 89 and 117% of CTR berry weight, with the exception of DMR. Overall, DMR produces lighter berries, MAD showed a lighter berry weight trend, and MED and MBT showed no marked difference from CTR. Generally, berry weight determined by berry sampling had higher weights than marked cluster determination and this can be attributed to the fact that berry sampling could only reach external berries (due to clusters compactness) missing the smaller internal berries, while marked cluster determination included all berries of the cluster.

Regarding pulp weight and skin weight, modalities showed generally lower skin weight than CTR and higher pulp weight than CTR, and so, apparently there was no gain in skin to pulp ratio when using the studied modalities. Pulp weight can be heavier if pulp has higher content of soluble solids, and this could explain the reason why berries from all modalities were heavier than CTR - they were more mature.

One other interesting result was the average seed number and seed number ratio with CTR. It is known that seed number can be related with higher viability of the berry [314,315], 2013 seed number results confirm that it was a difficult year and 2012 a more fertile and suitable year for the success and formation of berries. When using the ratio modality/CTR, MAD/CRT and MED/CRT ratio showed an increasing trend during the 3 vintages, while MBT/CRT and/ DMR/CRT ratio showed a decreasing trend. Apparently, MAD and MED showed increased berry viability when compared with CTR, and MBT and DMR showed lower berry viability. On the other hand, early leaf defoliation causes berry abortion and so less viable berries are not formed [314,315], and therefore these determinations were performed with the most viable berries from the clusters, the ones

that could strive after defoliation, naturally having higher seed number. This did not happen with MBT and DMR, which had, on average, a few more berries per clusters.

Table 24 - Averages values for modality influence over berry components - berry, pulp, skin and seed weight, skin/pulp ration, berry diameter and number of seeds per berry, for 2011, 2012 and 2013 vintages, determined using samples from 12 marked clusters per modality.

	Modality	2011 Averages	2012 Averages	2013 Averages	3-Vintage Average
Berry weight (g)	**CTR**	2.15	2.00	1.15	1.77
	MAD	1.95	2.00	1.00	1.65
	MED	2.15	2.20	1.35	1.90
	MBT	2.05	1.70	1.25	1.67
	DMR	1.60	1.60	0.40	1.20
Pulp weight (g)	**CTR**	0.95	0.85	0.50	0.77
	MAD	0.85	1.00	0.45	0.77
	MED	1.10	1.00	0.65	0.92
	MBT	1.10	0.70	0.60	0.80
	DMR	0.60	0.60	0.05	0.42
Skin weight (g)	**CTR**	0.90	0.95	0.50	0.78
	MAD	0.75	0.85	0.35	0.65
	MED	0.85	1.00	0.45	0.77
	MBT	0.85	0.80	0.50	0.72
	DMR	0.80	0.85	0.25	0.63
Skin/Berry (%)	**CTR**	42	48	43	44
	MAD	38	43	35	39
	MED	40	45	33	39
	MBT	41	47	40	43
	DMR	50	53	63	55
Skin/Pulp (%)	**CTR**	95	112	100	102
	MAD	88	85	78	84
	MED	77	100	69	82
	MBT	77	114	83	92
	DMR	133	142	500	258
Seed weight (g)	**CTR**	0.17	0.30	0.15	0.21
	MAD	0.19	0.30	0.20	0.23
	MED	0.15	0.30	0.25	0.23
	MBT	0.13	0.30	0.15	0.19
	DMR	0.11	0.30	0.10	0.17
Seed number	**CTR**	2.1	2.2	1.3	1.8
	MAD	1.6	3.0	1.5	2.0
	MED	2.0	2.6	2.2	2.3
	MBT	2.3	2.4	1.3	2.0
	DMR	2.3	2.1	1.1	1.8
Berry diameter (mm)	**CTR**	13.1	12.9	11.1	12.4
	MAD	13.9	13.4	10.2	12.5
	MED	13.7	13.6	12.5	13.3
	MBT	13.1	13.2	11.2	12.5
	DMR	11.9	11.6	6.9	10.1

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*'). n.a. - not available or not applicable.

Two remarks should be made about these results. First, Baga variety is traditionally described as a thin skin variety and this could explain why the early defoliation effect

over skin weight and skin to pulp ratio was not visible, while it was broadly mentioned in literature [228].

Table 25 - Modality to CTR ratio for each modality influence over berry components - berry, pulp, skin and seed weight, skin/pulp ration, berry diameter and number of seeds per berry, for 2011, 2012 and 2013 vintages, determined using 12 marked clusters per modality.

	Modality/CTR			
Modality	**2011 Averages**	**2012 Averages**	**2013 Averages**	**3-Vintage Average**
Berry weight (g)	n.a	n.a	n.a	n.a
	0.91	1.00	0.87	0.93
	1.00	1.10	1.17	1.09
	0.95	0.85	1.09	0.96
	0.74	0.80	0.35	0.63
Pulp weight (g)	n.a	n.a	n.a	n.a
	0.89	1.18	0.90	0.99
	1.16	1.18	1.30	1.21
	1.16	0.82	1.20	1.06
	0.63	0.71	0.10	0.48
Skin weight (g)	n.a	n.a	n.a	n.a
	0.83	0.89	0.70	0.81
	0.94	1.05	0.90	0.97
	0.94	0.84	1.00	0.93
	0.89	0.89	0.50	0.76
Skin/Berry (%)	n.a	n.a	n.a	n.a
	0.92	0.89	0.81	0.87
	0.94	0.96	0.77	0.89
	0.99	0.99	0.92	0.97
	1.19	1.12	1.44	1.25
Skin/Pulp (%)	n.a	n.a	n.a	n.a
	0.93	0.76	0.78	0.82
	0.82	0.89	0.69	0.80
	0.82	1.02	0.83	0.89
	1.41	1.27	5.00	2.56
Seed weight (g)	n.a	n.a	n.a	n.a
	1.13	1.00	1.33	1.16
	0.88	1.00	1.67	1.18
	0.78	1.00	1.00	0.93
	0.64	1.00	0.67	0.77
Seed number	n.a	n.a	n.a	n.a
	0.76	1.36	1.20	1.11
	0.98	1.18	1.76	1.31
	1.10	1.07	1.04	1.07
	1.12	0.95	0.88	0.99
Berry diameter (mm)	n.a	n.a	n.a	n.a
	1.06	1.04	0.91	1.00
	1.05	1.05	1.13	1.08
	1.00	1.02	1.01	1.01
	0.91	0.90	0.62	0.81

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*'). n.a. - not available or not applicable.

Second, early defoliation was reported previously to produce small berries [228] and results from this study did not showed the same trend. Again, the difficulties of berry sampling, with compact clusters, could explain these discrepancies from those reported in literature considering other varieties.

4.4.5 Yield

All studied modalities showed significantly lower yield when compared with CTR therefore succeeding the primary goal of the study (Table 26). In all 3 vintages, CTR yield was significantly higher than all modalities and no significantly differences were found within the other modalities, with the exception of DMR in 2013. The effect of lower yield for each modality was confirmed by these results. Though the expected lowering of yield for all studied modalities was confirmed, the reasons why these results occurred were not similar. MBT showed lower yield because the number of cluster per vine was inferior than CTR; MAD and MED had less berries; DMR has lighter berries.

Table 26 - Modality influence over yield per vine and average cluster weight, for 2011, 2012 and 2013 vintages, determined using samples from 12 marked clusters per modality. Average values and standard deviation (between brackets).

	Modality	2011 Averages	2012 Averages	2013 Averages	3-Vintage Average
Yield/vine (g)	CTR	4944.0 (2232.6) a	7425.4 (2097.9) b	4072.8 (1289.7) c	5480.7
	MAD	3373.6 (873.1) b	5532.1 (2031.9) a	2416.4 (1687.8) b	3774.0
	MED	3548.1 (1019.9) b	5435.4 (2526.7) a	2207.3 (1028.5) b	3730.3
	MBT	3215.7 (677.1) b	4618.8 (1736.1) a	2064.4 (517.5) b	3299.6
	DMR	3321.0 (1478.3) b	4645.5 (2394.0) a	742.0 (571.4) a	2902.8
	All Modality Average	3680.5	5531.4	2300.6	n.a.
Average Cluster weight (at harvest) (g)	CTR	274.8 (89.2) b	239.5 (79.4) b	173.4 (50.5) d	229.2
	MAD	171.3 (30.9) a	226.2 (101.5) ab	124.4 (46.3) bc	174.0
	MED	213.4 (71.1) a	158.2 (86.8) a	103.7 (43.4) ab	158.4
	MBT	190.7 (46.4) a	245.2 (77.5) b	145.3 (46.1) cd	193.7
	DMR	161.5 (44.0) a	239.0 (89.0) b	73.3 (26.3) a	157.9
	All Modality Average	202.3	221.6	124.0	n.a.

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*'). Different letters indicate significantly different averages, according to Duncan test $p<0.05$. n.a. - not available or not applicable.

The legal limit for grape production in Bairrada region is close to eleven tonnes per hectare [4] - knowing that there are an average of 3333 vines per hectare, the production for all modalities for 2011 and 2012 was in fact higher than this legal yield

limit. Only in 2013 all modalities achieved yield lower than the legal limit and CTR was above.

Table 27 - Modality influence over yield and yield per hectare, for 2011, 2012 and 2013 vintages, determined using samples from 12 marked clusters per modality.

	2011		2012		2013		3 Year
Modality	**Yield/vine**	**Yield/ha**	**Yield/vine**	**Yield/ha**	**Yield/vine**	**Yield/ha**	**Average/ha**
CTR	4944	16478	7425	24749	4073	13575	18267
MAD	3374	11244	5532	18438	2416	8054	12579
MED	3548	11826	5435	18116	2207	7357	12433
MBT	3216	10718	4619	15394	2064	6881	10998
DMR	3321	11069	4646	15483	742	2473	9675
Average	3680	12267	5531	18436	2301	7668	

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*').

Some differences were evident for the average cluster weight values: in 2011 vintage, CTR average cluster was significantly heavier than all the studied modalities, and this trend occurred in the subsequent vintages. No differences were found between the other modalities in 2011; in 2012, CTR, MBT and DMR clusters are significantly heavier than other modalities, with MED being the lowest weight modality and MAD in intermediate level. Also for 2012, DMR showed a heavier cluster weight when compared with 2011 and 2013 because the time period between DMR and harvest was 5 days, due to an earlier harvest. Overall, DMR, MAD and MED showed lower clusters weights, and MBT closer results to CTR, even though lighter.

As for leaf area-yield ratio, there are no significant differences, even though there are differences between the average values. Once again, the standard deviation is too high to have significant differences. The leaf area values were determined at *veraison*, when the removed leaf area for MAD and MED was already compensated; considering that values for leaf area to yield ratio below 5 cm^2/g are low and above 20 cm^2/g are high [38], the obtained values for all vintages and all modalities are intermediate.

Table 28 - Modality influence over yield, leaf area yield ratio and corrected leaf area yield ratio, for 2011, 2012 and 2013 vintages, determined using samples from 12 marked clusters per modality. Average values and standard deviation (between brackets).

	Modality	2011 Averages	2012 Averages	2013 Averages	3-Vintage Average
Yield/vine (g)	**CTR**	4944.0 (2232.6) a	7425.4 (2097.9) b	4072.8 (1289.7) c	5480.7
	MAD	3373.6 (873.1) b	5532.1 (2031.9) a	2416.4 (1687.8) b	3774.0
	MED	3548.1 (1019.9) b	5435.4 (2526.7) a	2207.3 (1028.5) b	3730.3
	MBT	3215.7 (677.1) b	4618.8 (1736.1) a	2064.4 (517.5) b	3299.6
	DMR	3321.0 (1478.3) b	4645.5 (2394.0) a	742.0 (571.4) a	2902.8
	All Modality Average	3680.5	5531.4	2300.6	n.a.
Leaf Area/Yield (cm^2/g)	**CTR**	5.18 (2.30) a	3.20 (1.20) a	7.79 (7.00) a	5.39
	MAD	6.04 (5.20) a	2.97 (1.30) a	7.18 (9.50) a	5.40
	MED	6.24 (1.40) a	4.40 (2.30) a	9.52 (12.40) a	6.72
	MBT	6.49 (3.50) a	5.45 (3.60) a	12.26 (6.80) a	8.07
	DMR	6.32 (5.00) a	5.53 (31.00) a	29.52 (66.90) b	13.79
	All Modality Average	6.05	4.31	13.25	n.a.
(Leaf Area x %GAPs)/Yield (cm^2/g)	**CTR**	4.68 (2.09) a	2.93 (26.68) a	6.59 (7.01) a	4.73
	MAD	5.53 (4.80) a	2.80 (28.82) a	5.69 (8.68) a	4.67
	MED	5.80 (1.42) a	4.09 (43.05) a	8.72 (12.65) a	6.20
	MBT	6.13 (3.76) a	4.69 (13.13) a	9.01 (9.34) a	6.61
	DMR	5.87 (4.66) a	4.83 (31.22) a	21.70 (56.48) b	10.80
	All Modality Average	5.60	3.87	10.34	n.a.

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*'). Different letters indicate significantly different averages, according to Duncan test $p<0.05$. n.a. - not available or not applicable.

4.4.6 Vegetative expression and vigour

Table 29 shows the variation of the pruning weight and the Ravaz index over the three vintages. The reference values for the Ravaz index vary between 5 and 12; 5-10 [39]; 5-7 [321]; 5-12 [322]. There are significant differences between modalities, mainly in 2013 - CTR where it is observed the highest pruning weight, and for MAD the value is quite similar, all other modalities had lower values. Nevertheless, these values have little interest because the vineyard owning company performed a shoot trimming in July, which masks these results.

Table 29 - Modality influence over pruning weight and Ravaz index, for 2011, 2012 and 2013 vintages, determined using samples from 12 marked clusters per modality. Average values and standard deviation (between brackets).

	Modality	2011 Averages	2012 Averages	2013 Averages	3-Vintage Average
Pruning weight/vine (g)	**CTR**	913.1 (491.3) a	904.8 (232.3) ab	895.0 (188.4) b	904.3
	MAD	771.4 (342.5) a	1046.0 (520.4) b	855.8 (193.6) ab	891.1
	MED	608.2 (207.6) a	683.3 (184.6) a	758.3 (191.7) ab	683.3
	MBT	795.6 (499.2) a	773.9 (266.0) a	775.0 (140.6) ab	781.5
	DMR	629.7 (151.8) a	745.5 (221.7) a	700.0 (218.5) a	691.7
	All Modality Average	743.6	830.7	796.8	n.a.
Ravaz Index	**CTR**	5.9 (2.2) a	7.8 (3.2) a	4.7 (1.8) c	6.1
	MAD	4.7 (1.5) a	5.7 (2.3) a	2.7 (1.5) b	4.4
	MED	6.2 (1.7) a	7.6 (3.3) a	3.1 (1.5) b	5.6
	MBT	5.0 (2.4) a	6.1 (2.3) a	2.5 (0.5) b	4.5
	DMR	5.4 (2.4) a	6.4 (3.1) a	1.1 (0.8) a	4.3
	All Modality Average	5.4	6.7	2.8	n.a.

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*'). Different letters indicate significantly different averages, according to Duncan test $p<0.05$. n.a. - not available or not applicable.

Regarding the Ravaz index, most of the values were in the lower part of the intervals suggested in literature and mentioned above, meaning that the yield-pruning ratio was small. Again, these results were affected by July shoot trimming operation and so the actual Ravaz index would be lower than the obtained values.

4.4.7 Fruit properties and quality

4.4.7.1 Ripening assessment

Grape ripening assessment was performed by sampling 200 berries once a week, during the three to four weeks prior to the expected harvest date - usually starting late August. Overall, the progression of grape ripening followed the expected behaviour, with the increase of total soluble solids (º Brix) and juice pH, and the decrease of the juice total titratable acidity (Table 30).

Table 30 - Modality influence over grape ripening - must soluble solids, pH and titratable acidity (TA), for 2011, 2012 and 2013 vintages, determined using 200 berries samples from marked clusters.

	Parameter	Modality	17/08/2011	26/08/2011	05/09/2011	16/09/2011
2011	Bloom until sampling (days)		105	114	124	135
	GDD		1332	1441	1543	1667
	Total soluble solids (º Brix)	CTR	16.9	18.5	18.0	19.0
		MAD	19.6	20.0	18.7	22.5
		MED	15.5	19.2	19.2	20.5
		MBT	16.4	20.0	19.2	19.5
		DMR	18.7	17.8	20.2	30.5
	Juice pH	CTR	3.02	2.99	3.15	3.40
		MAD	2.95	2.97	3.21	3.40
		MED	2.85	2.87	3.24	3.49
		MBT	2.96	3.00	3.18	3.48
		DMR	2.89	2.88	3.20	3.32
	Juice TA (g/L)	CTR	7.9	7.5	5.9	4.1
		MAD	7.7	7.3	5.8	4.6
		MED	8.5	8.0	5.8	4.7
		MBT	7.9	7.4	5.9	4.4
		DMR	8.4	8.1	7.0	6.9
	Parameter	**Modality**	**31/08/2012**	**07/09/2012**	**14/09/2012**	**19/09/2012**
2012	Bloom until sampling (days)		91	98	105	110
	GDD		1227	1323	1403	1461
	Total soluble solids (º Brix)	CTR	15.5	18.0	18.8	18.5
		MAD	14.4	16.4	19.4	20.0
		MED	13.3	16.6	18.2	17.5
		MBT	13.7	17.2	16.6	18.5
		DMR	13.3	15.6	18.0	21.0
	Juice pH	CTR	2.57	2.80	2.92	3.42
		MAD	2.55	2.73	2.95	3.33
		MED	2.59	2.75	2.93	3.30
		MBT	2.52	2.79	2.96	3.31
		DMR	2.52	2.79	3.00	3.45
	Juice TA (g/L)	CTR	10.1	9.2	7.1	4.4
		MAD	10.5	9.8	7.5	4.8
		MED	10.0	9.4	7.7	5.4
		MBT	11.1	9.8	8.3	4.9
		DMR	10.6	9.4	7.7	4.9
	Parameter	**Modality**	**30/08/2013**	**10/09/2013**	**19/09/2013**	**23/09/2013**
2013	Bloom until sampling (days)		86	97	106	110
	GDD		1245	1360	1447	1493
	Total soluble solids (º Brix)	CTR	16.0	17.2	20.0	21.0
		MAD	15.4	18.0	18.4	22.0
		MED	16.6	18.8	20.4	20.5
		MBT	17.2	18.0	20.8	21.0
		DMR	17.4	16.4	28.4	32.0
	Juice pH	CTR	2.80	3.06	3.18	3.53
		MAD	2.84	2.99	3.16	3.53
		MED	2.84	3.00	3.14	3.49
		MBT	2.78	2.92	3.13	3.59
		DMR	2.84	2.90	3.45	3.55
	Juice TA (g/L)	CTR	9.3	7.2	7.0	4.1
		MAD	8.9	6.7	6.2	4.3
		MED	8.6	6.8	6.4	4.5
		MBT	9.6	7.2	6.9	4.1
		DMR	9.0	7.0	7.1	6.3

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*').

Episodic rain events caused the deviations to these expected behaviours for soluble solids and acidity.

There were no significant relations between the ripening progressions of each modality when using as independent variable the number of days after blooming occurrence but there were some relations between ripening progression and the growing degree days (GDD) (Table 31).

Table 31 - Linear regression parameters between modality and GDD over grape ripening - must soluble solids, pH and titratable acidity (TA), for 2011, 2012 and 2013 vintages, determined using 200 berries samples from marked clusters.

		Slope	Intercept	r
Total soluble solids (º Brix) / GDD	**CTR**	0.00885	5.62	0.7063
	MAD	0.01645	-4.49	0.8457
	MED	0.01436	-2.25	0.8090
	MBT	0.01220	0.94	0.7262
	DMR	0.03672	-31.06	0.7501
Juice pH / GDD	**CTR**	0.00184	0.47	0.8124
	MAD	0.00193	0.33	0.8459
	MED	0.00203	0.18	0.8736
	MBT	0.00212	0.06	0.8698
	DMR	0.00194	0.32	0.7574
Juice TA / GDD	**CTR**	-0.01433	27.20	-0.8817
	MAD	-0.01376	26.43	-0.8582
	MED	-0.01258	24.91	-0.8614
	MBT	-0.01550	29.17	-0.8786
	DMR	-0.00859	19.83	-0.7083

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*').

Observing the slope and linear correlation coefficients (r) values for the estimated relations for total soluble solids, the studied modalities show faster ripening progressions than CTR, with noticeable linear correlations.

DMR showed higher values and it is explained by dehydration of the berries causing a sugar concentration progression much faster than natural ripening. Comparing the other modalities, MAD showed higher values than MED and both higher than MBT. It is reasonable to understand that MBT would increase global fruit ripening condition instantly because the greener part of the fruits might have been removed before; these results show that the ripening progression after MBT was faster than CTR, and so, the lower number of clusters might contribute to a faster ripening progression. As for leaf removal procedures, MAD and MED, higher ripening progressions were observed, with the highest values for MAD. The early removal of basal leaves and consequential lateral growth may explain these findings - basal leaves would be the oldest leaves during ripening and would have a small contribution (or none) to the fruit ripening; after

the early basal leaf removal, the laterals grow and they might contribute to grape ripening, representing a more active part of the canopy than principal leaves.

There were only small differences when comparing using pH and titratable acidity. The impact of temperature and climate over grape ripening might be more significant than the influence of the studied modalities, and this can explain the absence of differences when using acidity measurements.

These relations suggest that the modalities influenced the ripening speed - additional information if the impact was only over ripening speed or if it was also over ripening potential was not provided, that is, if the modalities could contribute for the fruits to achieve potential higher ripening degrees.

4.4.7.2 Botrytis incidence

Two other aspects were studied using the marked clusters - cluster compactness and the incidence of *Botrytis* (Table 32). It was observed that modalities had strong influence over cluster compactness in 2011 and 2013. MAD and MED contributed to loose clusters and DMR produced less clusters as well, with the lowest compactness index (average for DMR in 2012 was the lowest of all, even not being significant). MBT showed lower cluster compactness than CTR but not significantly different.

Table 32 - Modality influence over cluster compactness and *Botrytis* incidence, for 2011, 2012 and 2013 vintages, determined using samples from 12 marked clusters per modality. Average values and standard deviation (between brackets).

	Modality	2011 Averages	2012 Averages	2013 Averages	3-Vintage Average
Compactness (marked cluster)	**CTR**	6.8 (0.4) b	6.1 (1.7) a	7.5 (0.8) c	6.8
	MAD	5.2 (2.0) a	5.8 (1.3) a	5.9 (1.4) bc	5.6
	MED	6.4 (1.5) ab	5.8 (1.9) a	5.6 (2.8) b	5.9
	MBT	6.2 (0.8) ab	5.9 (1.6) a	6.9 (1.0) bc	6.3
	DMR	5.5 (2.0) ab	4.5 (0.8) a	3.9 (1.7) a	4.6
	All Modality Average	6.0	5.6	6.0	n.a.
Botrytis incidence (%) (marked cluster)	**CTR**	16.8 (8.1) a	6.4 (8.6) a	13.9 (13.8) b	12.4
	MAD	13.7 (9.2) a	3.3 (5.9) a	1.2 (3.9) a	6.1
	MED	11.5 (10.0) a	1.1 (3.8) a	3.9 (6.3) a	5.5
	MBT	17.8 (7.9) a	3.3 (5.9) a	15.7 (5.3) b	12.3
	DMR	13.7 (10.9) a	5.3 (8.5) a	5.8 (6.9) a	8.3
	All Modality Average	14.7	3.9	8.1	n.a.

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*'). Different letters indicate significantly different averages, according to Duncan test $p<0.05$. n.a. - not available or not applicable.

DMR showed lower compactness due to dehydrated berries, which retracted their size; MAD and MED were less compact as the number of berries was lower.

Regarding the *Botrytis* incidence in the marked clusters, only clusters from 2013 showed significant differences - standard deviations were too high for the differences in averages values to have some significance. Nevertheless, the trends observed for 2011 and 2012 vintages were confirmed with the significant results of 2013: MAD, MED and DMR showed lower *Botrytis* incidence in the clusters, and MBT displayed similar results as CTR. The looser clusters of MAD, MED and DMR were related with the lower incidence of *Botrytis* in these modalities and MBT showed no difference from CTR.

4.4.7.3 Fruit analysis at harvest

The last sampling of each vintage, occurring at harvest day, was used to perform a more extensive chemical analysis of the fruit, focusing also in polyphenolic content - colour and tannins.

Berry weight and volume are important parameters during the ripening, and they are disregarded most of the times. If berry volume increases during ripening it causes the dilution of the berry compounds. From the results of the three vintages (Table 33 and Table 34), it can be observed that MBT outcomes in an increase of berry volume compared with CTR; there was a similar behaviour for MED, so both MBT and MED might cause an increase of berry volume if performed. For all the vintages, MAD berries displayed to have lower volume than CTR, and DMR had a more broad decrease of berry volume. Higher berry volume results in a higher risk of berry skin rupture and subsequent *Botrytis* infection. In the particular case of MBT, the risk might be even higher because it will result in increased cluster compactness.

Regarding the sugar concentration, DMR and MAD showed higher sugar concentration/probable alcohol when compared with CTR; MED and MBT displayed values similar with CTR. One of the reasons that MAD and DMR show higher sugar concentrations is the lower berry volume, while MED and MBT presented higher berry volume.

As for acidity, titratable acidity and pH, the differences were small and should not have a perceptible effect in wine. Sugar load levels per berry were also calculated from sugar concentration and berry volume - it is understood that sugar load can be a

measure of ripening progression as it represents the amount of sugar present inside the berry.

It was observed that MAD, MED and MBT had similar or higher sugar load than CTR for 2011 and 2013 vintages, but lower values in 2012. As for DMR, even though sugar concentration is substantially higher than CTR for every vintage, the sugar load is lower than CTR in every vintage - this means that ripening didn't progress in DMR fruits as extensively as for CTR. This result was expected for DMR as the shoots and berries were disconnected from the vine in DMR and ripening was affected, even stopped. There is the hypothesis of ripening continuing to occur after cutting the shoots, but that would be possible only if the leaves would continue to produce photosynthates and the vessels transporting them to the fruits after being disconnected from the plant.

One important aspect regarding the usage of sugar load to determine the ripening progression is that this determination is not capable of distinguishing if the ripening would have occurred at a different rate (a higher sugar load at a determined sampling point represents that ripening occurred at a faster rate than the comparison value) or if the ripening have occurred in a different mechanism, occurring in different length (a higher sugar load at a determined sampling point represents that ripening occurred further than the comparison value).

Table 33 - Modality influence over must quality - berry weight and volume, soluble solids, pH and titratable acidity (TA), probable alcohol and sugar load per berry for 2011, 2012 and 2013 vintages, determined using 200 berries samples from marked clusters.

	Modality	Berries weight (g)	Juice volume	pH	Titratable acidity	º Brix	Probable Alcohol	Berry weight (g)	Berry volume	Sugar load (g/ berry)
2011	**CTR**	351	220	3.40	4.1	19.0	10.7	1.76	1.10	0.200
	MAD	326	218	3.40	4.6	22.5	13.1	1.63	1.09	0.241
	MED	390	270	3.49	4.7	20.5	11.8	1.95	1.35	0.267
	MBT	356	246	3.48	4.4	19.5	11.1	1.78	1.23	0.230
	DMR	189	94	3.32	6.9	30.5	18.7	0.95	0.47	0.153
2012	**CTR**	388	276	3.42	4.4	18.5	10.4	1.94	1.38	0.243
	MAD	373	230	3.33	4.8	20.0	11.4	1.87	1.15	0.222
	MED	364	256	3.30	5.4	17.5	9.7	1.82	1.28	0.211
	MBT	388	272	3.31	4.9	18.5	10.4	1.94	1.36	0.239
	DMR	293	188	3.45	4.9	21.0	12.1	1.47	0.94	0.192
2013	**CTR**	245	156	3.53	4.1	21.0	12.1	1.23	0.78	0.159
	MAD	239	150	3.53	4.3	22.0	12.8	1.20	0.75	0.161
	MED	251	164	3.49	4.5	20.5	11.8	1.26	0.82	0.162
	MBT	304	204	3.59	4.1	21.0	12.1	1.52	1.02	0.208
	DMR	164	76	3.55	6.3	32.0	19.8	0.82	0.38	0.131
Average	**CTR**	328	217	3.45	4.20	19.5	11.1	1.64	1.09	0.201
	MAD	313	199	3.42	4.57	21.5	12.4	1.56	1.00	0.208
	MED	335	230	3.43	4.87	19.5	11.1	1.68	1.15	0.214
	MBT	349	241	3.46	4.47	19.7	11.2	1.75	1.20	0.226
	DMR	215	119	3.44	6.03	27.8	16.9	1.08	0.60	0.159

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*').

Table 34 - Modality influence over wine quality - colour intensity (CI), anthocyanins concentration and per berry, total phenolics index (TPI) and HCl index for 2011, 2012 and 2013 vintages, determined using 200 berries samples from marked clusters.

	Modality	CI	Anthocyanins (mg/L)	Anthocyanins (mg/berry)	TPI (A280 nm)	HCl index
2011	CTR	11.860	337.5	371.3	32.6	15%
	MAD	13.690	410.4	447.3	36.4	13%
	MED	7.860	273.3	369.0	26.5	16%
	MBT	9.700	304.5	374.5	31.3	15%
	DMR	13.330	300.2	141.1	40.4	12%
2012	CTR	9.120	225.6	311.3	30.6	17%
	MAD	13.940	319.3	367.2	37.4	-7%
	MED	8.570	197.8	253.2	28.9	26%
	MBT	9.140	228.2	310.4	27.8	16%
	DMR	12.350	308.0	289.5	37.7	-17%
2013	CTR	15.480	367.0	286.3	43.9	19%
	MAD	17.900	391.3	293.5	50.0	19%
	MED	15.500	348.8	286.0	45.4	21%
	MBT	15.550	327.1	333.6	44.5	18%
	DMR	23.080	377.4	143.4	62.3	22%
Average	CTR	12.153	310.0	322.9	35.7	17%
	MAD	15.177	373.7	369.3	41.3	8%
	MED	10.643	273.3	302.7	33.6	21%
	MBT	11.463	286.6	339.5	34.5	16%
	DMR	16.253	328.5	191.3	46.8	6%

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*').

Colour intensity (CI) was higher for MAD and DMR comparing with CTR, and for MED and MBT was similar to lower than CTR. Anthocyanins concentration followed similar pattern, with higher values for MAD and DMR and lower values for MED and MBT, when compared with CTR. Colour intensity and anthocyanin concentration are concentration-related components and these results can be due to lower berry volume of MAD and DMR, and compound dilution in MED and MBT because of higher berry volume.

When observing the quantity of anthocyanins present per berry, MAD consistently showed higher values than CTR, the highest values of all modalities for 2011 and 2012 vintages. MAD favoured the formation of anthocyanins in the berry but MED did not, as its results were similar or lower than CTR. DMR showed the lowest values for each vintage - lower value for DMR might result from the ripening stoppage resulting from the shoot disconnection of the vine. MBT showed to have similar or higher amounts of anthocyanins than CTR (the highest value for 2013 vintage), showing that MBT might not favour the colour formation.

Total phenolics index (TPI) is a measurement of the phenolic compounds concentration present in the sample. MAD and DMR displayed regularly higher values than CTR, and MED and MBT displayed lower values than CTR in 2011 and 2012 vintages, having slightly higher values in 2013 vintage.

Hydrochloric acid index (HCl index) is based on the instability of procyanidins in a concentrated HCl medium, and precipitation rate depends on polymerization degree - the usual values for this index are between 5 and 40 [138]. A light wine at the beginning of barrel aging might have a low value, around 5 or 10. A wine suitable for ageing might have values ranged between 10 and 25 and a wine with a high concentration of highly polymerized phenolic compounds shows values above 25. For the wines that obtain index values above 35-40, the tannins in wine precipitate. Therefore, HCl index reflects the polymerization state of tannins in the wine, which also depends on the aging conditions. As an example, polymerization might decrease after winter cold or after fining, and also after some years in the bottle. MED showed to have higher polymerization degree than CTR, and MBT similar degree. As for MAD and DMR, it displayed lower values for 2011 and similar or higher for 2013 - the 2012 determinations displayed negative values, which imply that the samples show to have more stability after hydrochloric acid addition than in their natural state. These results were considered abnormal. In any case, the values obtained for this determination show that the grapes produced had potential to produce wines suitable for ageing.

4.4 Principal components analysis

Information is not completely translated by a single variable and therefore univariate data contains marginal information rather than complete information. We might be able to obtain a 'data structure' from observations and also 'noise', that can arise from other components, from the measurement, from the instrument, etc. The separation of the important data structure related to our study and the “noise” (information that has no significance as it is due to variations of instrumental signal and other processes) is a major problem when we are analyzing a set of observations, even because the data structure hides part of the information when we analyze only one variable.

With the aim to enlighten some more information about relations between viticultural aspects and the studied modalities, a Principal Components Analysis (PCA) was implemented. PCA was made using the inverse of standard deviation as scaling factor, followed by Cross validation, using Uncertainty test with optimal PC’s and a Full Size model.

Figure 16 shows the graph in which the different modalities and the viticultural variables were projected simultaneously according to PCA. The first three main components PC1, PC2 and PC3 explain 75% of the variance (46%, 17% and 12%, respectively). The first component (PC1) was predominantly characterized by the variables %Gaps, %IL and LA/Yield in the positive side of the axis, and Ravaz index, Yield, Cluster weight, and Berry weight in the negative side of the PC1. The second component (PC2) was characterized by the number of Berries, %Fruit set, LA and Cluster weight in the positive part of the PC2, and LLN, *Botrytis* incidence and Berry weight in the negative part of the PC2. The third component (PC3) was characterized by *Botrytis* incidence, Cluster compactness and LA in the positive part of the axis, and Bud break and Fertility indexes in the negative part (Figure 17). Regarding the samples/modalities of the study, PC1 was mainly defined by the modalities of the 2013 vintage in the positive part of the axis, and the modalities of the 2012 vintage in the negative part; PC2 by modalities CTR and MBT from 2012 and 2013 vintages in the positive part, and MAD, MED and DMR modalities from 2011 vintage in the negative part of the axis; finally, PC3 was characterized by modalities CTR and MBT from 2011 and 2013 vintages in the positive part, and MAD, MED and DMR modalities from 2012 and 2013 vintages in the negative part of the axis.

From these multivariate analysis results, a statistical relationship between variables, variables and modalities/samples, and between samples/modalities:

Using PC1, %Gaps, %IL and Leaf Area-Yield ratio were related, and also relate with the modalities of the 2013 vintage; Ravaz index, Yield, Cluster weight, and Berry weight were related and also relate with the modalities of the 2012 vintage;

From PC2, Berries, %Fruit set, LA and Cluster weight were related and also relate with modalities CTR and MBT from 2012 and 2013 vintages; LLN, *Botrytis* incidence and Berry weight were related and also relate with MAD, MED and DMR modalities from 2011 vintage;

Using PC3, *Botrytis* incidence, Cluster compactness and LA are related and also relate with modalities CTR and MBT from 2011 and 2013 vintages; Bud break and Fertility indexes are related and also relate with MAD, MED and DMR modalities from 2012 and 2013 vintages.

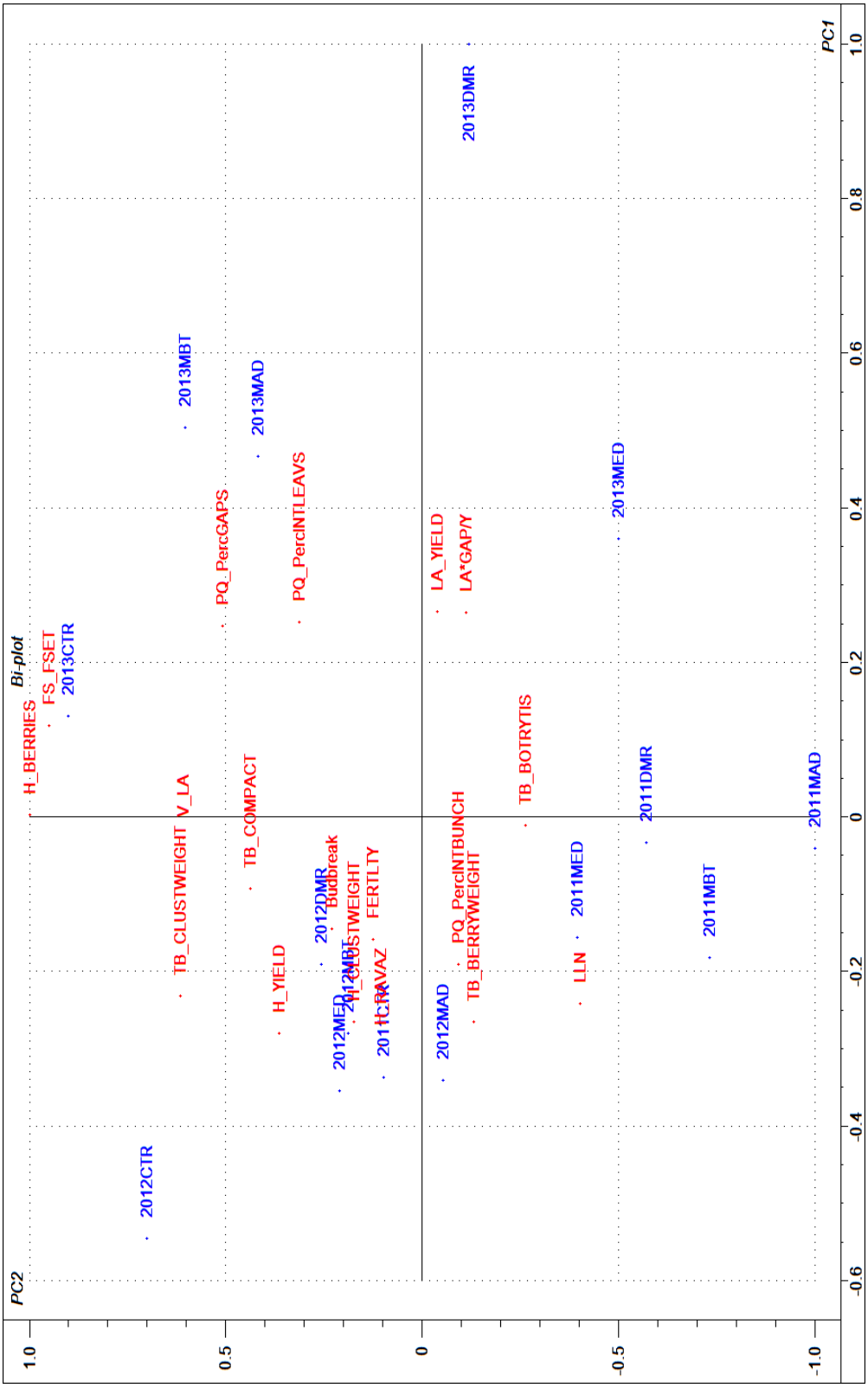

Figure 16 - Principal components analysis (PCA) Scores and Loadings Biplot of viticultural variables and modalities (PC1 46%; PC2 17% of explained variance).

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR (‘*Double maturation raisonnée*’).

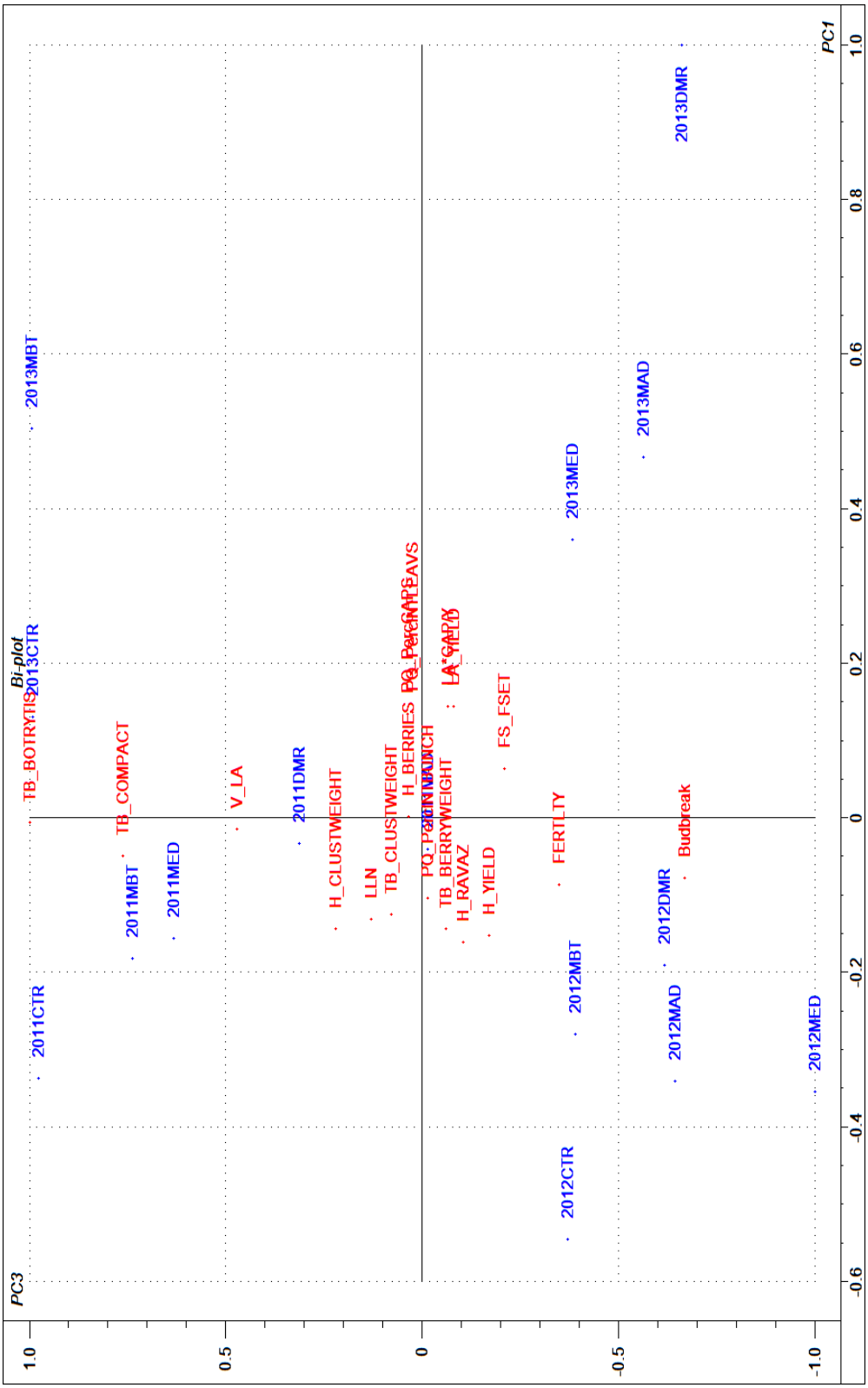

Figure 17 - Principal components analysis (PCA) Scores and Loadings Biplot of viticultural variables and modalities (PC1 46%; PC3 12% of explained variance).

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR (‘*Double maturation raisonnée*’).

Figure 18 shows the graph in which the different modalities and the Berry components variables were projected simultaneously. The first three main components PC1, PC2 and PC3 explain 94% of the variance (72%, 13% and 09%, respectively). The first component (PC1) was characterized only by the variable Skin-pulp ratio in the positive side of the axis, and all the remaining variables showed similar importance in the negative side of the axis. The second component (PC2) was characterized by the Seed weight, Seed number and Skin-pulp ratio in the positive part of the axis, and Pulp weight in the negative part of the axis. The third component (PC3), Figure 19, was characterized by Skin-pulp ratio and Skin weight in the positive part of the axis, and Seed weight in the negative part. Regarding the samples/modalities of the study, PC1 was mainly defined by the modalities of the 2013 vintage in the positive part of the axis, and the modalities of the 2012 and 2011 vintage in the negative part; PC2 by modalities from 2012 vintage in the positive part, and modalities from 2011 vintage and CTR and MBT from 2013 vintage in the negative part of the axis; finally, PC3 was characterized by DMR samples and modalities from 2011 and 2012 vintages in the positive part, and modalities from 2013 vintage in the negative part of the axis.

From these multivariate analysis results, we might point a statistical relation between variables, variables and modalities/samples, and between samples/modalities:

Using PC1, Skin-pulp ratio was related with the modalities of the 2013 vintage; modalities of the 2012 and 2011 vintage were related between them;

From PC2, Seed weight, Seed number and Skin-pulp ratio were related and also relate with modalities from 2012 vintage; Pulp weight relate with modalities from 2011 vintage and CTR and MBT from 2013 vintage;

Using PC3, Skin-pulp ratio and Skin weight were related and also relate with DMR samples and modalities from 2011 and 2012 vintages; Seed weight relate with modalities from 2013 vintage.

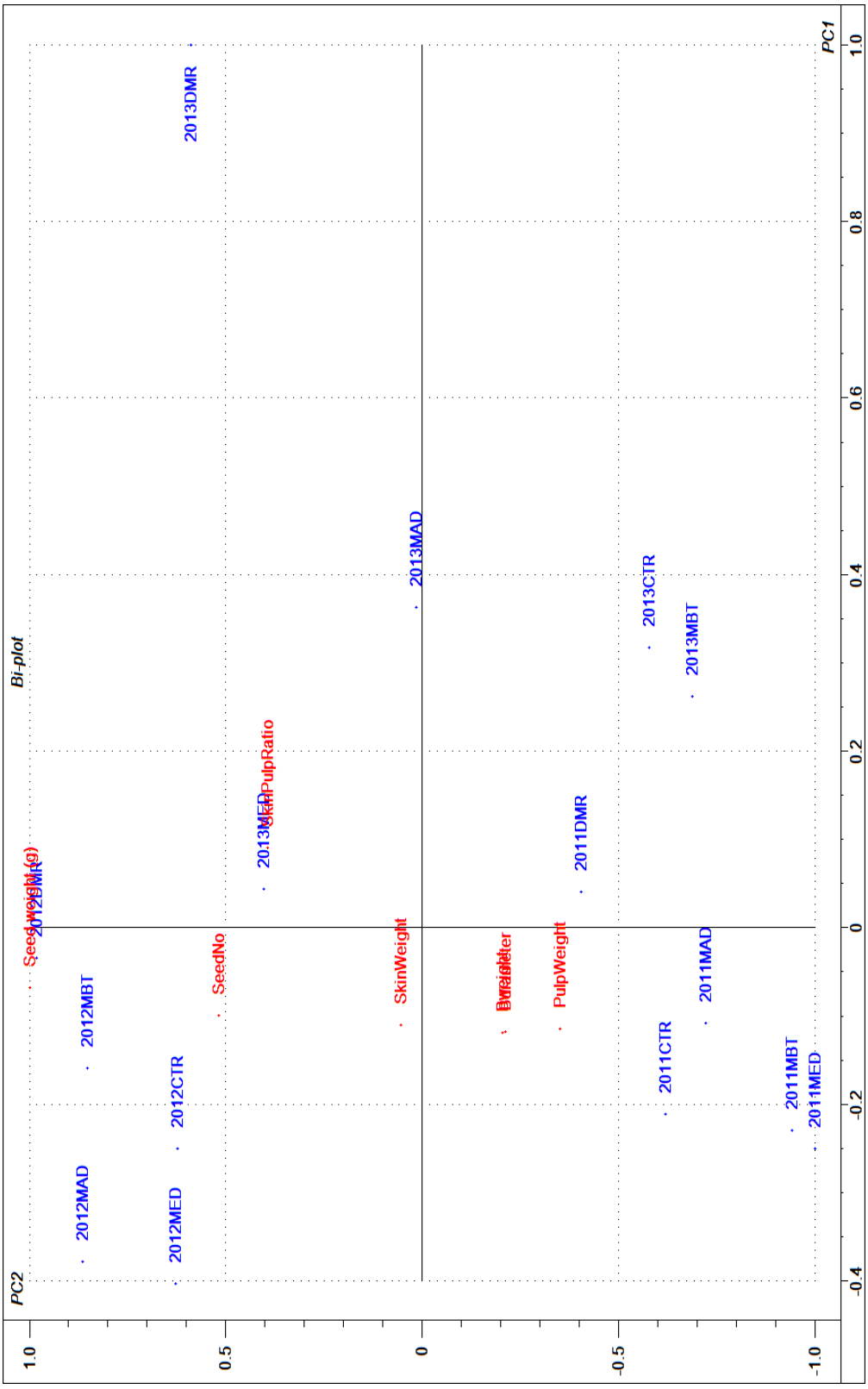

Figure 18 - Principal components analysis (PCA) Scores and Loadings Biplot of Berry components and modalities (PC1 72%; PC2 13% of explained variance).

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*').

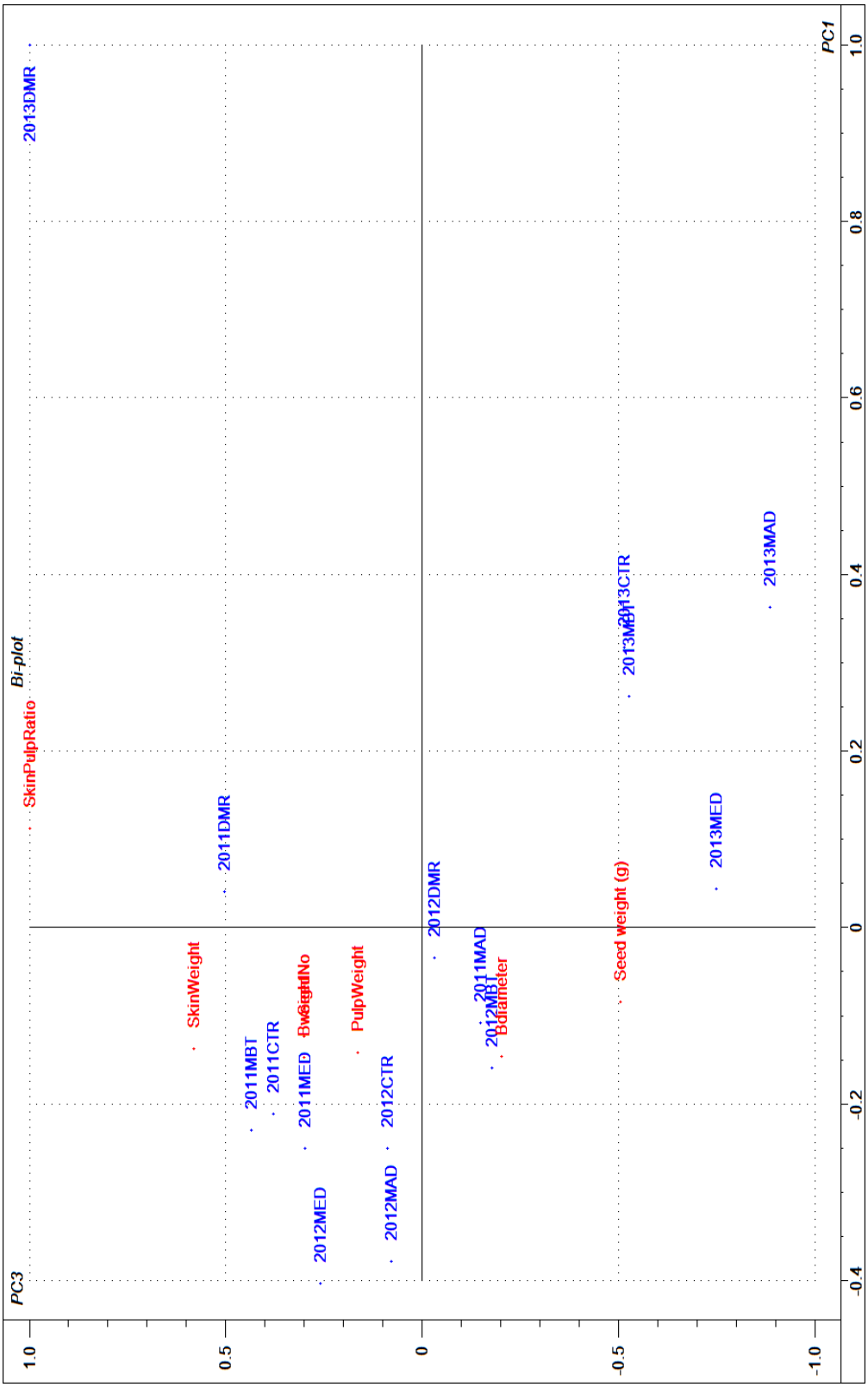

Figure 19 - Principal components analysis (PCA) Scores and Loadings Biplot of Berry components and modalities (PC1 72%; PC3 09% of explained variance).

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*').

Figure 20 shows the graph in which the different modalities and the ripening variables were projected simultaneously according to PCA. The first three main components PC1, PC2 and PC3 explain 90% of the variance (67%, 15% and 8%, respectively). The first component (PC1) was predominantly characterized by the variables ºBrix and spectroscopic variables (colour, IPT, etc.) in the positive side of the axis, Berry volume, Sugar load and Berry weight in the negative side of the axis. The second component (PC2) was characterized by the pH and Anthocyanins concentration in the positive part of the axis, and Titratable acidity and ºBrix in the negative part of the axis. The third component (PC3) was characterized by TC, HCl index and pH in the positive part of the axis, and Anthocyanins concentration in the negative part (Figure 21). Regarding the samples/modalities of the study, PC1 was mainly defined by the DMR and modalities of the 2013 vintage in the positive part of the axis, and the modalities of the 2012 and 2011 vintages in the negative part; PC2 by modalities from 2013 vintage in the positive part, and DMR and modalities from 2012 and 2011 vintages in the negative part of the axis; finally, PC3 was characterized by modalities MED and MBT in the positive part, and MAD and DMR modalities in the negative part of the axis.

From these multivariate analysis results, we might point a statistical relationship between variables, variables and modalities/samples, and between samples/modalities:

Using PC1, º Brix and spectroscopic variables were related, and also relate with the DMR and modalities of the 2013 vintage; Berry volume, Sugar load and Berry weight were related and also relate with the modalities of the 2012 and 2011 vintages;

From PC2, pH and Anthocyanins concentration were related and also relate with modalities from 2013 vintage; Titratable acidity and º Brix were related and also relate with DMR and modalities from 2012 and 2011 vintages;

Using PC3, TC, HCl index and pH are related and also relate with modalities MED and MBT; Anthocyanins concentration relate with MAD and DMR modalities.

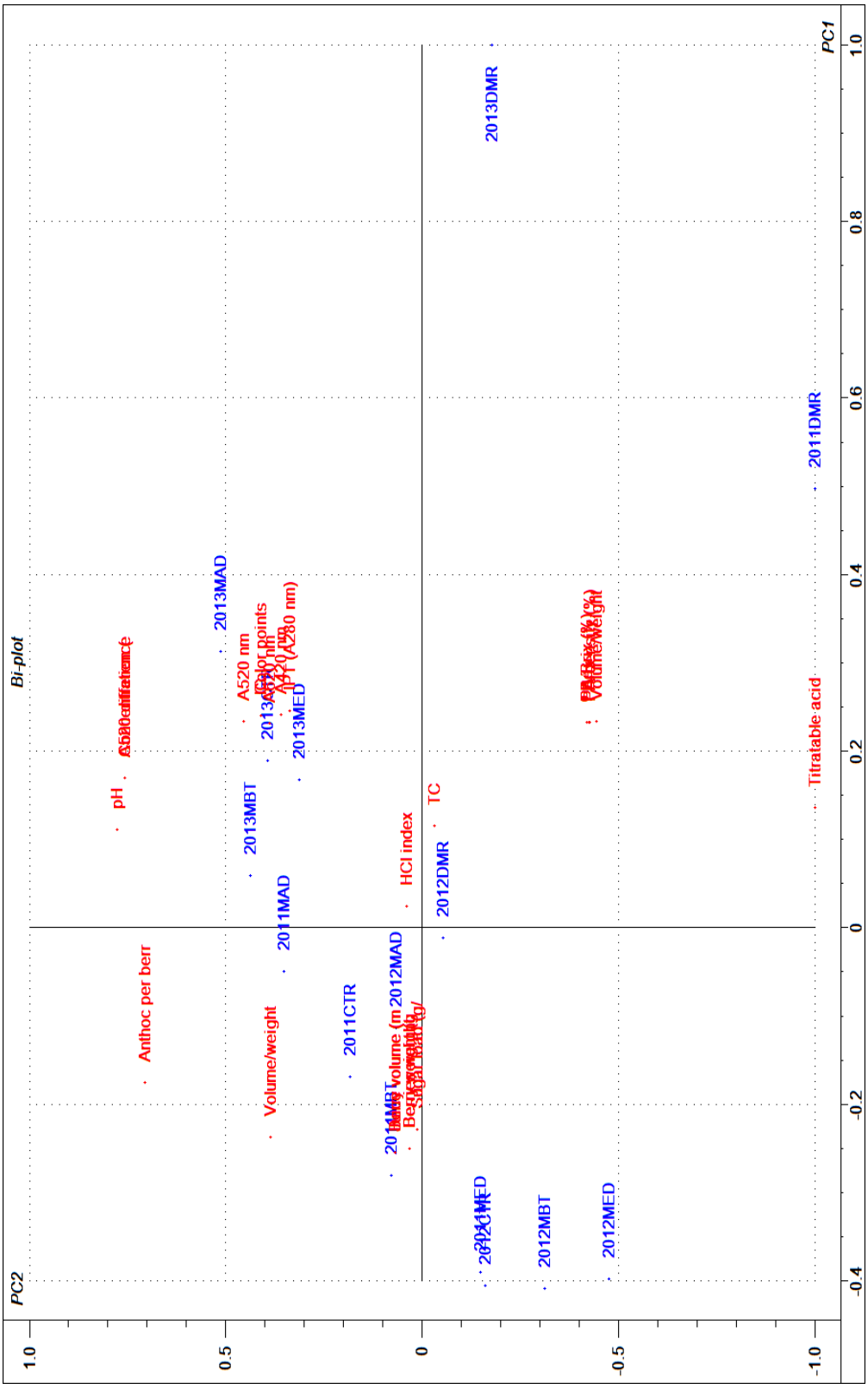

Figure 20 - Principal components analysis (PCA) Scores and Loadings Biplot of ripening variables and modalities (PC1 67%; PC2 15% of explained variance).

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*').

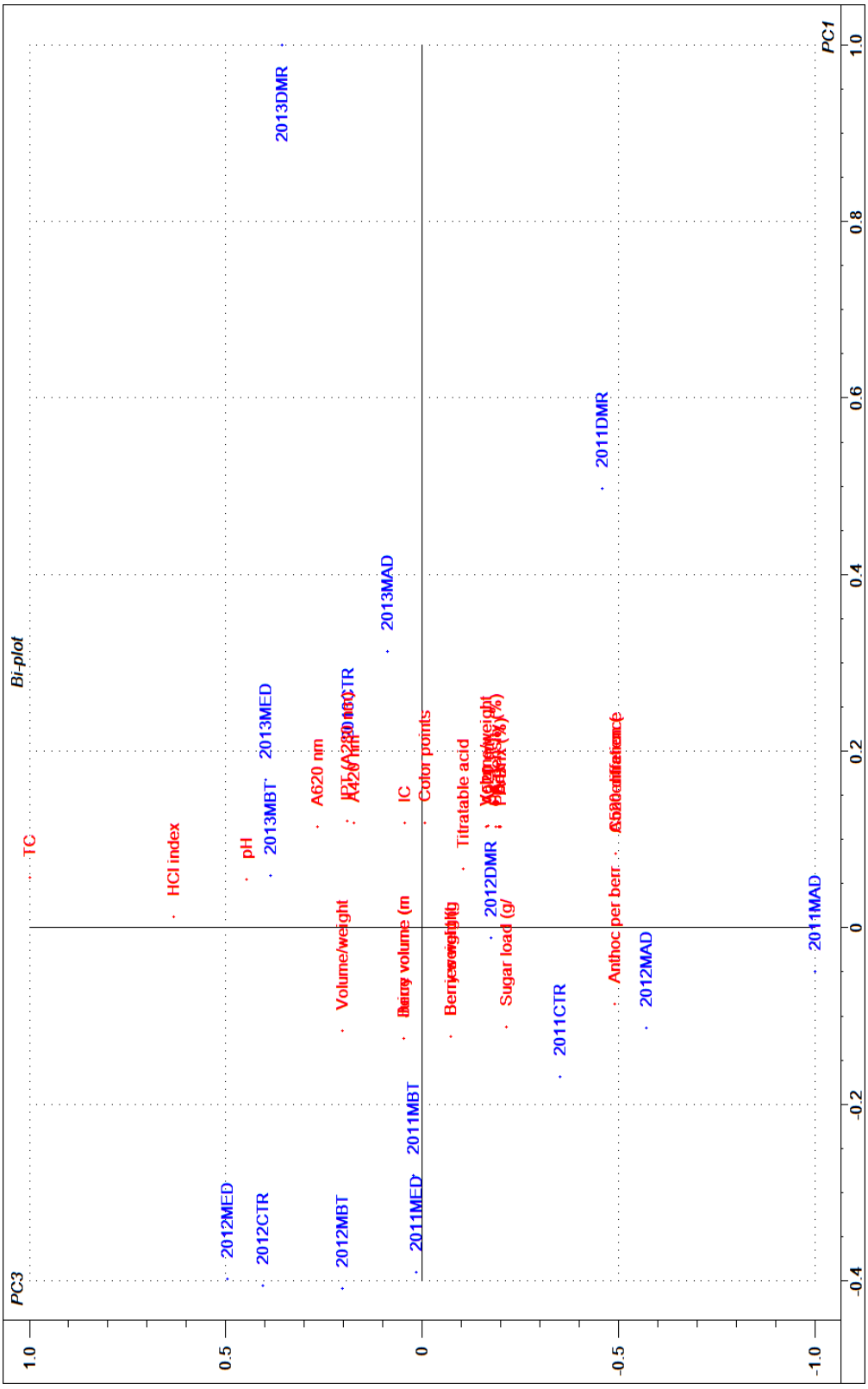

Figure 21 - Principal components analysis (PCA) Scores and Loadings Biplot of ripening variables and modalities (PC1 67%; PC3 8% of explained variance).

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*').

V Carotenoids and chlorophylls - Results and discussion

5.1 Introduction

Carotenoid degradation reactions may occur during the ripening of the grapes, with the consequent norisoprenoid formation and their contribution for juice and wine aroma. Their impact might be relevant to the wine aroma, depending on the viticultural conditions that influence carotenoid profile and content, and subsequent degradation during ripening and wine production.

The chlorophyll function in photosynthesis is known and it was suggested that this compound class has effect in occurrence of berry sunburn symptoms [323]. On other hand, carotenoids perform critical and complementary functions, collecting light and photo-protection [324] - they are able to quench chlorophylls on their excited states, releasing the energy in form of heat. Chlorophyll and carotenoid have also important function in grape pigmentation, especially in white grape, were anthocyanins are not present. Following events of extreme radiation and oxidative stress, some of the common occurrences are chlorophyll loss, formation of brown areas or even tissues death [323,325]. Small damages, associated with small chlorophyll losses, might be resolved by the plant mechanisms. However, there might be conditions when the light damage exceeds the plant protection capability and might result in tissues damage and chlorophyll degradation.

5.1 Carotenoid and chlorophylls content

The determination of carotenoid compounds was performed using samples of previously frozen grapes, below -32º C. The quantified compounds were antheraxanthin, β-carotene, chlorophyll a, chlorophyll b, lutein, neoxanthin, pheophytin, violaxanthin and zeaxanthin. Antheraxanthin, chlorophyll a, neoxanthin, violaxanthin and zeaxanthin exhibited analytical responses below the detection limit of the method (< 1.5 μg/g dry weight).

The obtained results were divided in two tables: Table 35 and Table 36 display the amounts of carotenoids and chlorophylls per berry and also the concentration per dry weight, respectively. The amount present per berry is useful for viticulture studies because it is independent from the dilution effect if some rain occurs, or a long drought period. The concentration per dry weight is important for winemaking studies because represents the conditions to be used to produce wine. It is important to have in consideration that the amount of each compound present in the berry contributes to the concentration that could be present in the wine but the contribution is not proportional to the berry concentration because of the extraction of the compounds from the grapes.

As mentioned above and as expected, β-carotene and lutein were the major carotenoids detected, with all other being not detected in all samples, for all vintages.

When observing the carotenoid and chlorophylls content per berry (Table 35), similar average values for total carotenoids were found for 2011 and 2012 vintages, with lower value for 2013 vintage; as for average values of total chlorophylls, the highest value was found in 2012, with the lowest value found in 2013.

β-Carotene displayed averages with highest value for 2012, with 2011 content being a slightly smaller and 2013 even smaller. CTR displayed similar values for all three vintages, slightly above 400 μg per berry; MAD showed lower content than CTR for 2011 and 2013, and higher for 2012; MED presented values smaller than CTR for all vintages, the same behaviour as DMR; MBT showed smaller values than CTR for 2011 and 2013 vintages, and higher for 2012. Overall, CTR had the highest values in 2011 and 2013, MAD alternated between the lowest value in 2011 and 2013 and the highest value in 2012; and MED, MBT and DMR displayed similar values through the vintages. Regarding the 3-vintage averages for all modalities, β-carotene displayed similar values with only small differences, and CTR having the highest content of β-carotene.

Table 35 - Carotenoid and chlorophylls content per berry, for 2011, 2012 and 2013 vintages Average values and standard error (between brackets).

	Modality	2011 Averages	2012 Averages	2013 Averages	3-Vintage Average
β-carotene (μg/berry)	CTR	0.459 (0.021)	0.449 (0.022)	0.419 (0.023)	0.442
	MAD	0.375 (0.031)	0.522 (0.029)	0.307 (0.012)	0.401
	MED	0.434 (0.019)	0.350 (0.027)	0.358 (0.016)	0.381
	MBT	0.380 (0.020)	0.476 (0.018)	0.325 (0.003)	0.394
	DMR	0.425 (0.020)	0.424 (0.024)	0.302 (0.024)	0.384
	DMR30	n.q.	n.q.	0.254 (0.019)	n.a.
	BIO	n.q.	n.q.	0.382 (0.009)	n.a.
	Average	0.415	0.444	0.342	n.a.
lutein (μg/berry)	CTR	1.890 (0.027)	1.980 (0.054)	0.910 (0.031)	1.593
	MAD	1.820 (0.132)	1.780 (0.072)	0.910 (0.027)	1.503
	MED	1.840 (0.049)	1.580 (0.041)	1.210 (0.055)	1.543
	MBT	1.630 (0.043)	1.840 (0.051)	1.150 (0.039)	1.540
	DMR	1.680 (0.042)	1.750 (0.047)	1.360 (0.052)	1.597
	DMR30	n.q.	n.q.	0.780 (0.045)	n.a.
	BIO	n.q.	n.q.	0.890 (0.050)	n.a.
	Average	1.772	1.786	1.108	n.a.
chlorophyll b (μg/berry)	CTR	0.810 (0.103)	1.360 (0.129)	0.320 (0.027)	0.830
	MAD	1.060 (0.125)	0.810 (0.071)	0.270 (0.024)	0.713
	MED	1.180 (0.103)	1.060 (0.099)	0.390 (0.032)	0.877
	MBT	1.050 (0.094)	1.200 (0.101)	0.370 (0.041)	0.873
	DMR	0.960 (0.095)	0.870 (0.076)	0.470 (0.037)	0.767
	DMR30	n.q.	n.q.	0.280 (0.032)	n.a.
	BIO	n.q.	n.q.	0.370 (0.031)	n.a.
	Average	1.012	1.060	0.364	n.a.
pheophytin (μg/berry)	CTR	0.782 (0.047)	1.033 (0.092)	0.844 (0.057)	0.886
	MAD	1.263 (0.039)	1.440 (0.092)	1.067 (0.075)	1.257
	MED	0.946 (0.079)	0.929 (0.079)	1.077 (0.080)	0.984
	MBT	0.804 (0.057)	0.827 (0.063)	1.132 (0.106)	0.921
	DMR	0.780 (0.062)	1.055 (0.089)	0.844 (0.064)	0.893
	DMR30	n.q.	n.q.	0.549 (0.045)	n.a.
	BIO	n.q.	n.q.	0.519 (0.044)	n.a.
	Average	0.915	1.057	0.993	n.a.
Total carotenoids (μg/berry)	CTR	2.349	2.429	1.329	2.036
	MAD	2.195	2.302	1.217	1.905
	MED	2.274	1.930	1.568	1.924
	MBT	2.010	2.316	1.475	1.934
	DMR	2.105	2.174	1.662	1.980
	DMR30	n.q.	n.q.	1.034	n.a.
	BIO	n.q.	n.q.	1.272	n.a.
	Average	2.187	2.230	1.450	n.a.
Total chlorophylls (μg/berry)	CTR	1.592	2.393	1.164	1.716
	MAD	2.323	2.250	1.337	1.970
	MED	2.126	1.989	1.467	1.861
	MBT	1.854	2.027	1.502	1.794
	DMR	1.740	1.925	1.314	1.660
	DMR30	n.q.	n.q.	1.034	n.a.
	BIO	n.q.	n.q.	1.272	n.a.
	Average	1.927	2.117	1.357	n.a.

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*'). Antheraxanthin, chlorophyll a, neoxanthin, violaxanthin and zeaxanthin had concentrations below the detection limit of the method (< 1.5 μg/g dry weight).n.q. - not quantified. n.a. - not available.

Average content in lutein per berry had similar values for 2011 and 2012, differing for 2013 - this behaviour was more or less similar in all studied modalities. CTR displayed close values for 2011 and 2012 (around 1.9 μg per berry), and a significantly smaller value for 2013; MAD displayed smaller or equal values than CTR for all vintages; MED, MBT and DMR showed similar behaviour, with smaller values than CTR for 2011 and 2012, and higher values for 2013. CTR showed the highest lutein content between all modalities for 2011 and 2012 vintages, and the lowest for 2013; MBT displayed the smallest for 2011, MED for 2012 and CTR and MAD for 2013. Regarding the 3-vintage averages for all modalities, lutein displayed similar values (between 1.5 and 1.6 μg per berry) with MBT and CTR having the highest content and MAD the lowest.

Concerning the chlorophyll b, vintage averages showed higher and similar content per berry for 2011 and 2012 vintages, and a significantly lower value for 2013. CTR chlorophyll b content increased from 2011 to 2012, and 2013 was much lower than the previous vintages. MAD showed lower values than CTR for 2011 and 2013, and higher for 2012; MED, MBT and DMR displayed higher values for 2011 and 2013 and lower for 2012. The highest values for each vintage were for MED for 2011, CTR for 2012 and DMR for 2013, and the lowest CTR for 2011 and MAD for 2012 and 2013 vintages. About the 3-vintage averages for all modalities, chlorophyll b displayed values between 0.7 and 0.9 μg per berry, with MED and MBT having the highest content and MAD the lowest.

Pheophytin displayed a more consistent trend through the vintages, showing similar vintage averages values. CTR pheophytin content increased from 2011 to 2012 vintage, and decreased again in 2013. MAD showed higher values than CTR for all vintages; MED and MBT displayed higher values than CTR for 2011 and 2013 and lower values for 2012; DMR pheophytin content was similar to CTR for all vintages. Comparing the modalities for each vintage, the highest values for all vintages were for MAD, with CTR and DMR showing the lowest values for 2011 and 2013, and MBT for 2012. Regarding the 3-vintage averages for all modalities, CTR, MED, MBT and DMR displayed similar values (around 0.9 μg per berry) with MAD having the highest content of all.

Beside the content per berry, the concentration of carotenoids and chlorophylls can also have important information to understand the impact of the modalities over fruit qualities (Table 36). β-Carotene vintage averages increase from 2011 until 2013 and concentration in CTR samples followed the same trend. MAD displayed higher

concentrations than CTR for 2011 and 2012 and lower for 2013; MED presented higher concentration than CTR for 2011 and lower for 2012 and 2013; MBT showed lower concentrations than CTR in 2011 and 2013, and higher in 2012; and DMR displayed lower concentrations than CTR in all vintages. Regarding the 3-vintage averages for all modalities, CTR displayed highest concentration and DMR the lowest.

Table 36 - Carotenoid and chlorophylls content per dry weight of skin, for 2011, 2012 and 2013 vintages. Average values and standard error (between brackets).

	Modality	2011 Averages	2012 Averages	2013 Averages	3-Vintage Average
β-carotene (μg/g DW)	CTR	12.63 (0.59)	15.20 (0.74)	22.46 (1.24)	16.76
	MAD	13.89 (1.13)	16.56 (0.92)	17.30 (0.70)	15.92
	MED	14.46 (0.64)	12.88 (1.01)	14.02 (0.63)	13.79
	MBT	11.23 (0.60)	15.52 (0.59)	16.54 (0.17)	14.43
	DMR	12.37 (0.58)	13.61 (0.78)	11.07 (0.87)	12.35
	DMR30	n.q.	n.q.	10.97 (0.83)	n.a.
	BIO	n.q.	n.q.	17.97 (0.43)	n.a.
	Average	12.92	14.75	16.28	n.a.
lutein (μg/g DW)	CTR	51.85 (0.76)	66.94 (1.84)	48.83 (1.65)	55.87
	MAD	67.43 (4.90)	56.37 (2.30)	51.34 (1.53)	58.38
	MED	61.14 (1.62)	58.13 (1.50)	47.35 (2.13)	55.54
	MBT	48.18 (1.29)	59.99 (1.65)	58.56 (2.00)	55.58
	DMR	48.82 (1.21)	56.12 (1.50)	49.78 (1.90)	51.57
	DMR30	n.q.	n.q.	33.58 (1.94)	n.a.
	BIO	n.q.	n.q.	41.75 (2.34)	n.a.
	Average	55.48	59.51	51.17	n.a.
chlorophyll b (μg/g DW)	CTR	22.41 (2.82)	45.96 (4.39)	17.17 (1.47)	28.51
	MAD	39.43 (4.62)	25.64 (2.26)	15.00 (1.37)	26.69
	MED	39.22 (3.42)	38.85 (3.63)	15.20 (1.25)	31.09
	MBT	30.93 (2.77)	39.05 (3.28)	19.01 (2.09)	29.66
	DMR	28.04 (2.77)	27.87 (2.42)	17.38 (1.36)	24.43
	DMR30	n.q.	n.q.	12.08 (1.40)	n.a.
	BIO	n.q.	n.q.	17.40 (1.48)	n.a.
	Average	32.01	35.47	16.75	n.a.
pheophytin (μg/g DW)	CTR	21.50 (1.30)	34.98 (3.11)	45.18 (3.04)	33.89
	MAD	46.79 (1.44)	45.83 (2.93)	60.10 (4.24)	50.91
	MED	31.50 (2.61)	34.20 (2.90)	42.15 (3.12)	35.95
	MBT	23.78 (1.68)	26.97 (2.06)	57.58 (5.41)	36.11
	DMR	22.71 (1.81)	33.84 (2.84)	30.94 (2.35)	29.16
	DMR30	n.q.	n.q.	23.68 (1.95)	n.a.
	BIO	n.q.	n.q.	24.43 (2.08)	n.a.
	Average	29.26	35.16	47.19	n.a.

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*'). Antheraxanthin, chlorophyll a, neoxanthin, violaxanthin and zeaxanthin had concentrations below the detection limit of the method (< 1.5 μg/g dry weight). n.q. - not quantified. n.a. - not available.

Lutein average concentrations increase from 2011 to 2012 and dropped for 2013, with CTR having the same tendency. MAD showed higher concentrations than CTR in 2011 and 2013, and lower in 2012; MED displayed higher concentrations than CTR in 2011 and lower for 2012 and 2013; MBT and DMR presented lower concentrations

than CTR for 2011 and 2012 and higher 2013. Concerning the 3-vintage averages, MAD had the highest average and DMR the lowest.

As for the chlorophylls, chlorophyll b concentration increased from 2011 to 2012 and lowered for 2013, and concentration for CTR samples followed the same trend. MAD and MED displayed higher concentrations than CTR for 2011 and lower for 2012 and 2013; MBT and DMR showed higher concentrations than CTR for 2011 and 2013 and lower for 2012. Regarding the 3-vintage averages, MED had the highest average and DMR the lowest.

Finally, pheophytin concentrations increased from 2011 until 2013, with CTR following the same behaviour. MAD showed higher concentrations than CTR for all vintages; MED displayed higher concentration than CTR for 2011 and lower for 2012 and 2013; MBT presented higher concentrations than CTR for 2011 and 2013 and lower for 2012; DMR showed higher concentration than CTR for 2011 and lower for 2012 and 2013. Concerning the 3-vintage averages, MAD had the highest average and DMR the lowest.

Regarding the violaxanthin, antheraxanthin, and zeaxanthin xanthophylls carotenoids (VAZ) that act like non-photochemical quenchers, these compounds were not present at harvest time. This behaviour could be due to earlier degradation, earlier than the rest of quantified carotenoids.

Lutein and β-carotene were cited as skin protection compounds [326] and so, their content might be strongly related with cluster sunlight exposure but light exposure also is associated with faster temperature-related degradation. On another hand, carotenoids are formed and then degraded during ripening, alcoholic fermentation and wine conservation phases. The found values do not provide conclusions because carotenoid act as intermediates and there was only a sampling moment, not being possible to ascertain the magnitude of carotenoid formation before *veraison* and the extent of degradation during ripening until the harvest moment (and also the extent of norisoprenoid formation).

The determinations were performed using berries collected at harvest time so these results are not able to present a broad insight about carotenoids content behaviour. It is known that carotenoids are formed during berry formation until *veraison*, and degradation begins to occur after this moment [98]. Because of using one-point sampling timing, it is not possible to know the total amount formed for each carotenoid, the extent of degradation that was occurring until the sampling moment and how the extension of degradation contributed to aromatic compound formation.

The carotenoid content for CTR was higher than any other studied modality, for β-carotene and for lutein. These results might be associated with the faster ripening mechanism observed for all modalities other than CTR and so, the lower carotenoid content might be due to faster degradation of these compounds.

Since the carotenoid content determinations were performed in berries and not in wines, there was not possible to observe the higher-age/lower-carotenoid trend mentioned above.

Observing the chlorophylls content for the 3-vintage average, all values were similar and close except for MAD. MAD displayed the lowest value for chlorophyll b content, and the highest value for pheophytin. Knowing that chlorophylls function in the plant, the premature and prolonged light exposure of the clusters due to MAD might explain difference of behaviour of MAD when compared with the other modalities. The higher total content in chlorophylls of MAD and MED might help to agree with this finding.

DMR showed to have the lowest 3-year average concentration of chlorophylls, displaying consistently low values through the vintages. One might expect high values for DMR because of dehydration, which enables the concentration of the compounds present. On the other hand, degradation might also occur faster because of the reducing biological functions of the shoots and leaves due to the cutting of the shoot.

Chlorophyll b content for 2013 was substantially lower than the other vintages. The lower vegetative development of the plants in this vintage might explain the lower content, because of having less leaves to protect the clusters and having a higher risk of light exposure skin damage.

It may be interesting to quantify the evolution of chlorophylls from cluster formation until harvest with the aim to determine if Baga variety is more or less susceptible to light damage and if early light exposure of the cluster due to leaf removal induces change in chlorophylls mechanisms.

5.3 Principal components analysis

With the aim to enlighten some more information about relationship between carotenoid composition and the studied modalities, a Principal Components Analysis was performed. Again, PCA was performed using the inverse of standard deviation as scaling factor, followed by cross validation, using uncertainty test with optimal PC's and a full size model.

Figure 22 displays the graph in which the different modalities and the carotenoid content per berry were projected simultaneously according to PCA. The first three main components PC1, PC2 and PC3 explain 98% of the variance (60%, 26% and 12%, respectively). The first component (PC1) contained the variables in the positive side of the axis. The second component (PC2) was characterized by the pheophytin and β-carotene content in the positive part of the axis, and chlorophyll b content in the negative part of the axis. The third component (PC3) was characterized by β-carotene content in the positive part of the axis, and chlorophyll b, lutein and pheophytin content in the negative part (Figure 23). Regarding the samples/modalities of the study, PC1 was mainly defined by the modalities of the 2012 and 2011 vintages in the positive part of the axis, and the modalities of the 2013 vintage in the negative part; PC2 by MAD and 2013 vintage modalities in the positive part, and CTR and MBT modalities in the negative part of the axis; finally, PC3 was characterized by CTR from 2013 vintage in the positive part, and MAD, MED and DMR modalities from 2012 and 2013 vintages in the negative part of the axis.

From these multivariate analysis results, we might point a statistical relation between variables, variables and modalities/samples, and between samples/modalities:

Using PC1, all variables were related, and also relate with modalities of the 2012 and 2011 vintages; modalities of the 2013 vintage had no direct positive relation with other variables or samples;

From PC2, pheophytin and β-carotene content were related and also relate with MAD and 2013 vintage modalities; chlorophyll b content relate with CTR and MBT modalities;

Using PC3, β-carotene content relate with CTR from 2013 vintage; chlorophyll b, lutein and pheophytin content were related and also relate with MAD, MED and DMR modalities from 2012 and 2013 vintages.

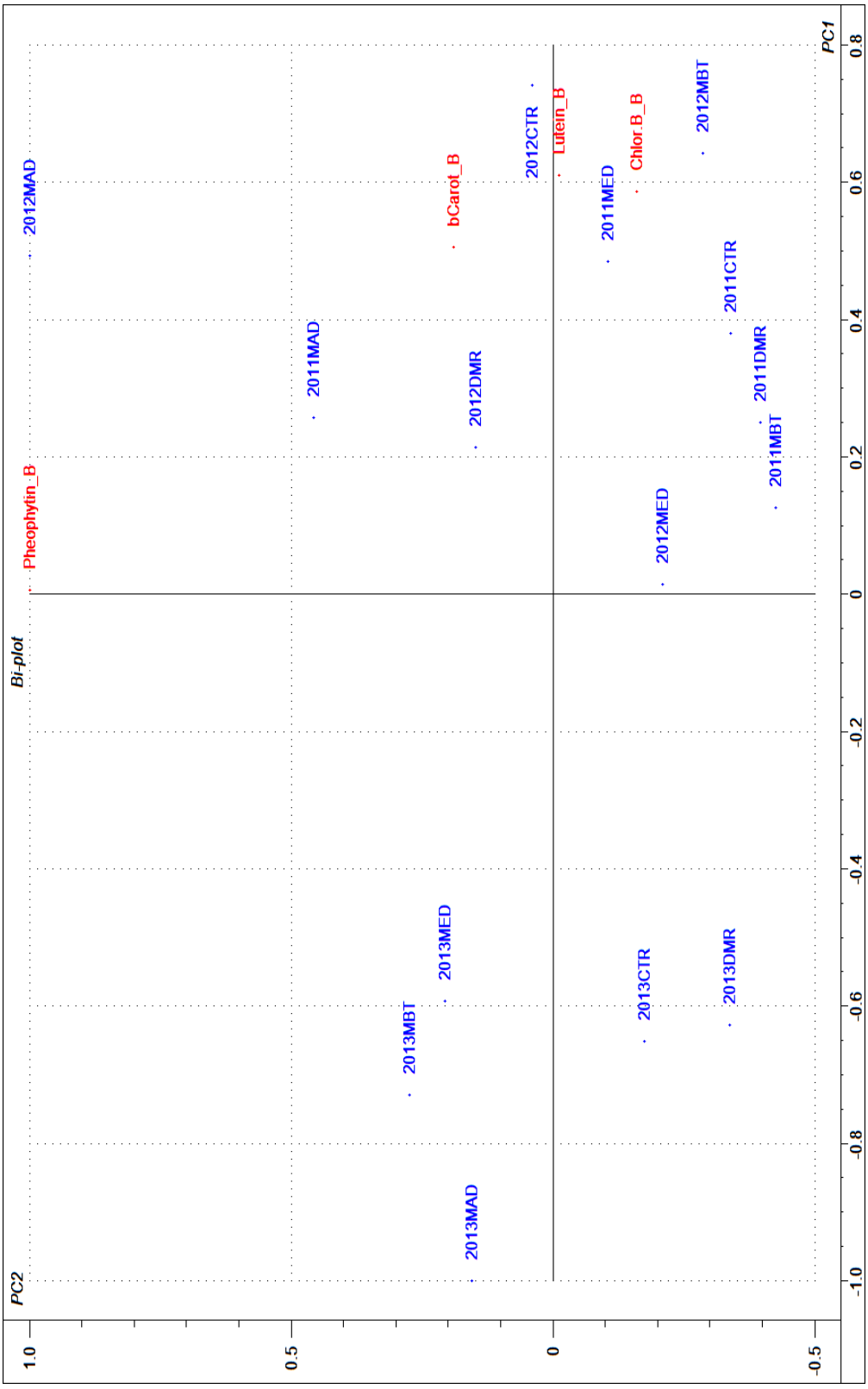

Figure 22 - Principal components analysis (PCA) Scores and Loadings Biplot of carotenoid content per berry variables and modalities (PC1 60%; PC2 26% of explained variance).

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*').

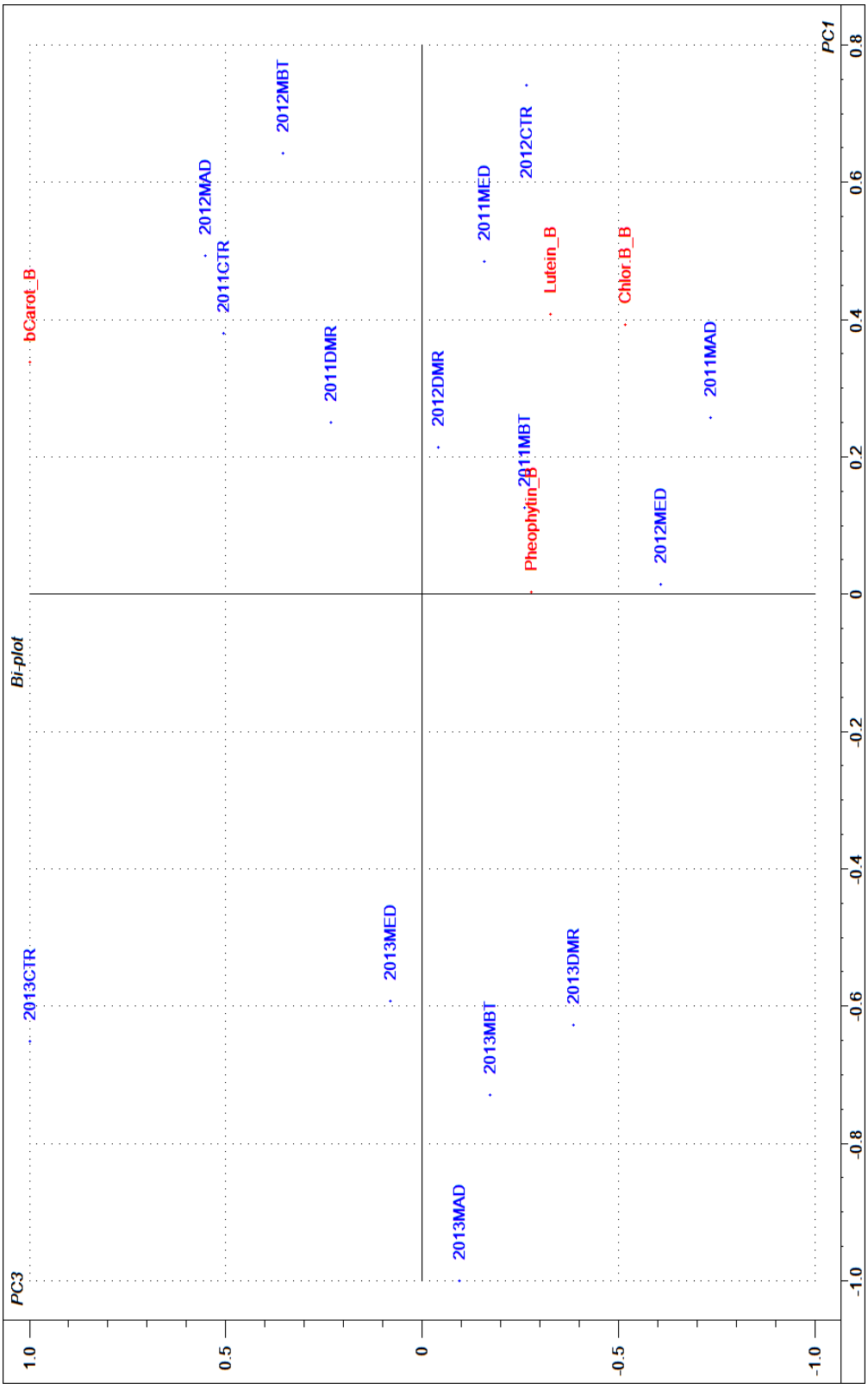

Figure 23 - Principal components analysis (PCA) Scores and Loadings Biplot of carotenoid content per berry variables and modalities (PC1 60%; PC3 12% of explained variance).

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*').

Figure 24 shows the graph in which the different modalities and the carotenoid concentration were projected simultaneously according to PCA. The first three main components PC1, PC2 and PC3 explain 99% of the variance (49%, 39% and 11%, respectively). The first component (PC1) was predominantly characterized by the chlorophyll b and lutein concentration in the positive side of the axis, and pheophytin and β-carotene concentration in the negative side of the axis. The second component (PC2) was characterized by all variables in the positive part of the axis. The third component (PC3) was characterized by β-carotene and chlorophyll b concentration in the positive part of the axis, and pheophytin and lutein concentration in the negative part (Figure 25). Regarding the samples/modalities of the study, PC1 was mainly defined by the modalities of the 2012 and 2011 vintages in the positive part of the axis, and the modalities of the 2013 vintage in the negative part; PC2 by modalities MAD and MED in the positive part, and DMR and modalities from 2011 vintage in the negative part of the axis; finally, PC3 was characterized by CTR modalities in the positive part, and modalities from 2013 vintage in the negative part of the axis.

From these multivariate analysis results, we might point a statistical relation between variables, variables and modalities/samples, and between samples/modalities:

Using PC1, chlorophyll b and lutein concentration were related, and also relate with the modalities of the 2012 and 2011 vintages; pheophytin and β-carotene concentration were related and also relate with the modalities of the 2013 vintage;

From PC2, all variables were related and also relate with modalities MAD and MED; DMR and modalities from 2011 vintage were related and were represented in the negative part of the axis;

Using PC3, β-carotene and chlorophyll b concentration were related and also relate with CTR modalities; pheophytin and lutein concentration are related and also relate with modalities from 2013 vintage.

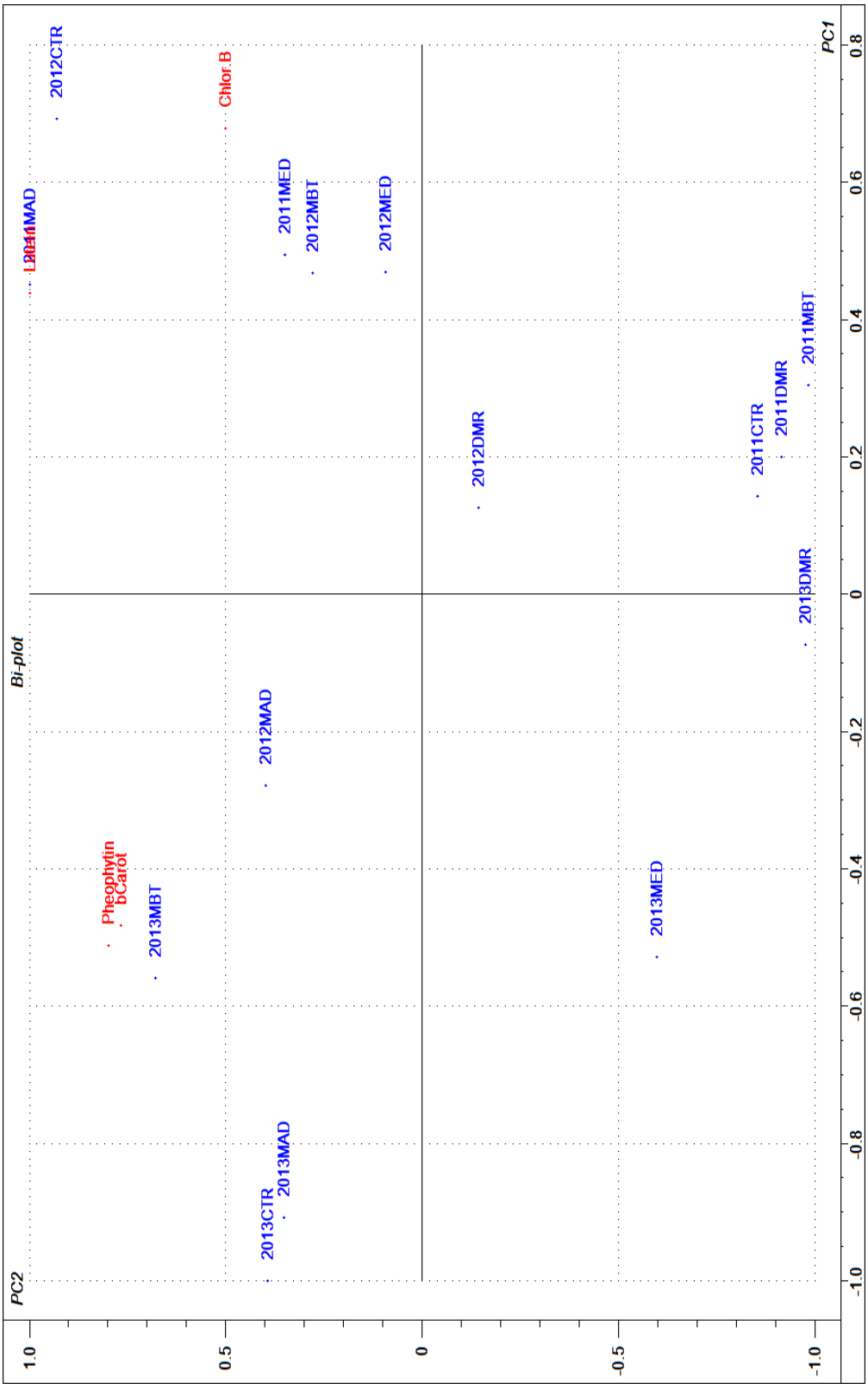

Figure 24 - Principal components analysis (PCA) Scores and Loadings Biplot of carotenoid concentration variables and modalities (PC1 49%; PC2 39% of explained variance).

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*').

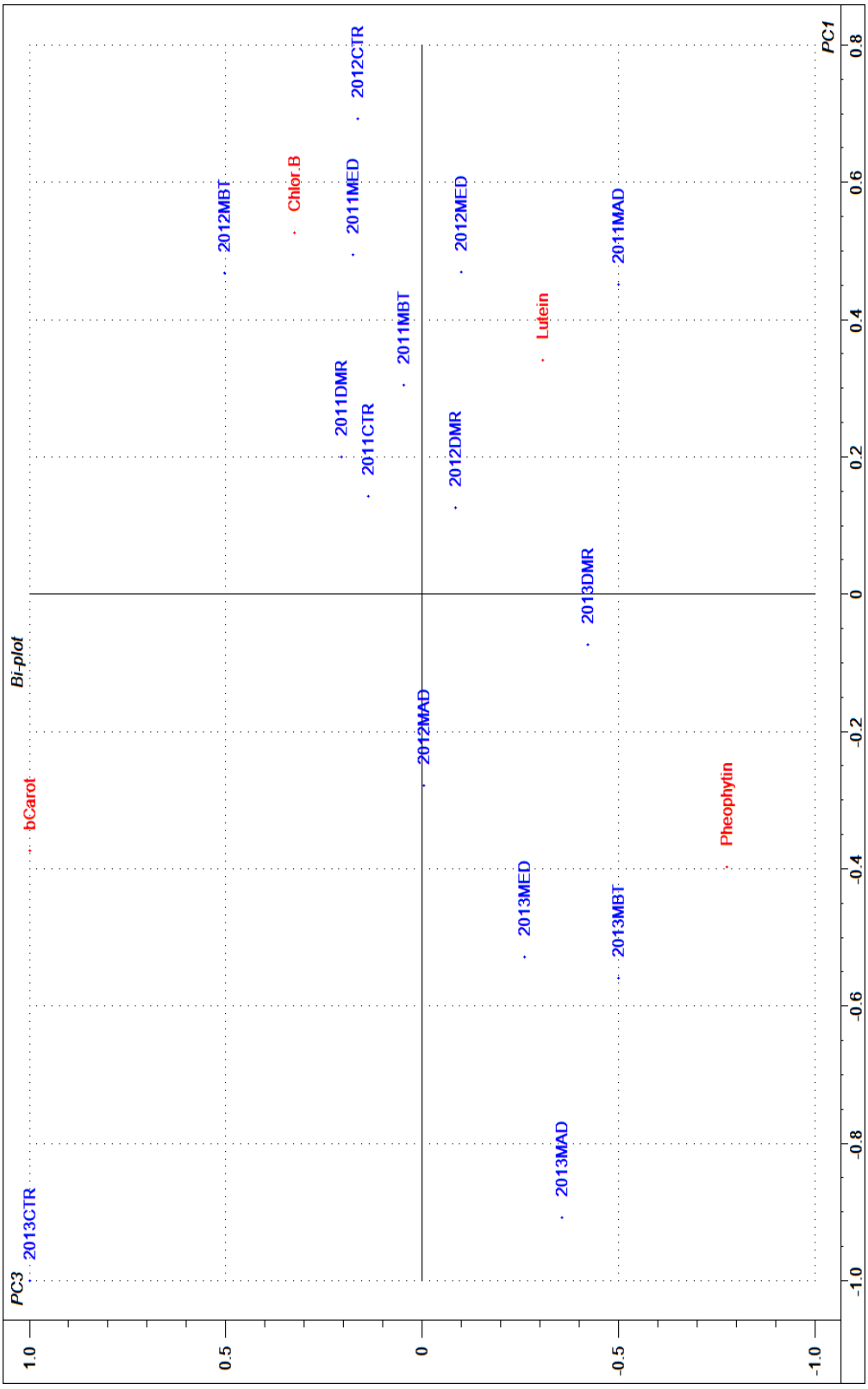

Figure 25 - Principal components analysis (PCA) Scores and Loadings Biplot of carotenoid concentration variables and modalities (PC1 49%; PC3 11% of explained variance).

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*').

VI Aroma composition of Baga wines - Results and discussion

6.1 Introduction

Unfortunately, there are only a few of papers regarding Baga varietal aroma and wine composition and none of the existing papers had an organized sensorial analysis or used a tasting panel.

The first work studying Baga aroma composition reported a total of 53 compounds identified and quantified [179] in wines, with the majority being aliphatic and aromatic alcohols, acids, esters, and small quantities of lactones, amides and phenols. Of these identified compounds, nine were recognized to be the odorants with higher impact: guaiacol, 3-methylbutanoic acid, 4-ethoxycarbonyl-γ-butyrolactone, isobutyric acid, 2-phenylethanol, γ-nonalactone, octanoic acid, ethyl octanoate and 4-(1-hydroxyethyl)-γ-butyrolactone.

Other research work [180] focused on the evolution of aroma compounds in grapes of Baga variety during ripening and monitored the ripening for 7 weeks, since *veraison* until full ripening. A significant number of sesquiterpenoids, monoterpenoids and norisoprenoids were identified during the ripening, with the authors concluding that sesquiterpenoids were an important group for grape aroma of Baga. These data suggest that Baga grapes have a fruity-type aroma correlated to a restricted number of compounds. The identified and quantified compounds were not similar to the ones described in the first work [179].

Apart from these works, little more information regarding Baga aromatic content was found and none on scientific research papers. Another important aspect to be mentioned is that it is regionally accepted that Baga wines need some time in wood barrel before being ready for consumption, so it is difficult to find Baga wines commercially available that did not have been a period of time in barrel [312].

Apart from carotenoids, there are other compounds able to participate in adaptive mechanisms of the grapevine to sun light. Phenolic compounds are key secondary metabolites present in grapevine leaves and, principally, in the skins and berries' seeds. They are responsible for most of the sensorial quality aspects of grape and wine composition, such as colour, taste and mouthfeel [327]. Phenolic compounds may have the role of filtering UV radiation and act as antioxidants. Their accumulation in leaves and berries is one of the most important adaptive mechanisms of grapevine to UV light [328,329].

6.2 Aromatic compound content

The evaluation of the aromatic compounds has the objective of identifying and quantifying these substances present in the wines in each vintage and from grapes originated from the vines subject to different canopy management techniques: Manual early leaf removal (MAD), Mechanical early leaf removal (MED), Manual bunch thinning (MBT) and '*Double maturation raisonnée*' (DMR), compared with the Control samples (CTR). Grapes were destemmed and crushed after arriving at the 'winery', and inoculated at once, in order to prevent any delay in starting the alcoholic fermentation. After finishing fermentation, a small volume of sulphur dioxide solution was added to each individual wine/modality, enough to achieve a concentration of 90 mg/L. No other products were added to the must and/or wines.

The wines were analysed at the same time, in 2014 (harvest, fermentation and wine conservation is described in Chapter III).

Wines from 2011 and 2012 had significant quantities of volatile acidity (ethyl acetate and acetic acid) which affected the sensory perception of the other compounds as well as the chromatographic feature of each wine extract. Probably due to this aspect, several compounds were only identified and quantified in the 2013 wines, and others were only identified in 2011 and 2012 wines. Some commercial wine (some included barrel ageing during their production) and DMR30 and BIO wines were also analysed and their results were included for comparison with the studied modalities.

The list of volatile compounds identified using the SPME-GC-MS method [307] in monovarietal Baga red wines are shown in Table 37. The list also includes odour descriptors, odorant series and odour threshold for each compound. A total of 40 compounds were quantified, and 34 were identified as well, including ethyl esters, acetates, alcohols, terpenes, sesquiterpenes, norisoprenoids and volatile phenols.

The largest groups of compounds identified and quantified were esters and terpenes. Table 38 displays esters concentrations in the wines from the study and several commercially obtained wine references (only for comparison) and Table 39 shows esters analytical chromatographic areas' in the wines from the study and several commercial wine references. Even though the chromatographic areas do not provide information about the concentration of these compounds in each sample, it could still provide some information about how do every modality influences grape and/or wine quality in each vintage.

Table 37 - Compounds quantified by SPME-GC-MS method, odour descriptors, odorant series and odour threshold.

Compound	Odour threshold (µg/L)	Odour descriptors	Odorant series
ethyl butanoate (mg/l)	**20**	Papaya, pineapple, fruity, juicy, fruit.	1
ethyl hexanoate (mg/l)	**14**	Apple peel, pineapple, fruity, waxy, estery, green banana	1, 3, 4, 7
ethyl heptanoate (mg/l)	**22**	Fruity, pineapple, sweet, estery, banana, strawberry, cognac, green, spicy, oily	1, 3, 4, 5, 7
ethyl octanoate (mg/l)	**50 - 600**	Fruity, sweet, pear, pineapple, banana, apricot, fat, waxy, musty, wine, mushroom	1, 2, 3, 4, 6, 7
ethyl decanoate (mg/l)	**23 - 200**	Grape, apple, dry fruit, solvent, oily	1, 7
ethyl dodecanoate (mg/l)	**400 - 1500**	Sweet, waxy, soapy, rum, cream, floral	1, 2, 6, 7
ethyl 2-methyl-butanoate (A/10^7)	**18**	Apple, fruity, fruity, fresh, berry, grape, pineapple, mango, cherry	1, 3
ethyl 3-methyl-butanoate (A/10^7)	**3**	Apple, fruity, pineapple, green, orange, sipce	1, 3, 5
ethyl *trans*-4-decenoate (A/10^7)	not available	Green, fruity, oily, pineapple, apple, waxy	1, 3, 7
***iso*amyl acetate (mg/l)**	**30**	Banana, estery, apple	1, 3, 7
phenylethyl acetate (mg/l)	**250**	Rose, honey, Sweet, floral, yeasty, honey,cocoa, balsamic	2, 3, 4, 7
diethyl succianate (A/10^7)	**200000**	Cheese, earthy, spicy, cooked apple, ylang	2, 5, 6, 7
***iso*amyl hexanoate (A/10^7)**	**30**	Fruity, sweet, pineapple, pungent, sour cheese	1, 4, 6, 7
phenylethyl alcohol (mg/l)	**10000 - 14000**	Rose, lilac, bread, honey	2, 4, 7
benzyl alcohol (A/10^7)	**200000**	Flowery, sweet, rose, phenolic, balsamic	2, 3, 4, 7
α-terpineol (A/10^7)	**400**	Floral, citrus, sweet, pine, lilac, woody	1, 2, 3, 7
β-*cis*-terpineol (A/10^7)	**400**	Pungent, earthy, woody	3, 7
β - linalol (mg/l)	**25**	Muscat, citrus, fresh, floral, lavender, sweet, waxy	1, 2, 3, 4, 7
nerolidol (A/10^7)	**700 - 2250 - 10000**	Floral, green, citrus, woody, waxy	1, 2, 3, 7
limonene (µg/l)	**200**	Lemon, orange, citrus	1
terpinolene (A/10^7)	**0.2**	Citrus, Fresh, Pine, Plastic, Sweet, Woody	1, 3, 4, 7
s-α-pinene (A/10^7)	**0.006**	Pine, resin, turpentine	3, 7
geranyl acetone (µg/l)	**186**	Earth, Fatty, Floral, Fresh, Fruit, Green, Herbaceous, Magnolia, Meat, Nut, Rose, Spicy, Tropical, Wax, Wine	1, 2, 3, 5, 6, 7
neryl Acetate (µg/l)	**880 - 905400**	Floral, Rose, Fruity, Raspberry	1, 2
***cis*-β-farnesene (A/10^7)**	not available	Green apple, gardenia, green, citrus, woody	1, 2, 3, 7
***trans*-α-bisabolene (A/10^7)**	not available	Balsamic, oregano, citrus	1, 5, 7
***trans*-nerolidol (A/10^7)**	**700**	Green, floral, woody, fruity, citrus, melon	1, 2, 3, 7
4-ethylguaiacol (A/10^7)	**0.05 - 33**	Spicy, clove-like medicinal, woody, sweet vanilla, animal, barnyard, stable, phenolic, mousy	2, 5, 7
4-ethylphenol (A/10^7)	**440**	Smoke, savory, animal, barnyard, stable, phenolic, and mousy	2, 5, 7
dihydro pseudo-ionone (A/10^7)	not available	Sweet, waxy, citrus, floral, balsamic, dry, dusty, powdery, spicy	1, 2, 4, 5, 7
β-damascenone (µg/l)	**0.05**	Natural sweet, fruity, rose, plum, grape, raspberry, apple, honey, sugar, smoky, tobacco	1, 2, 4, 7
TDN (A/10^7)	**2**	Licorice, petrol	5, 7
ethyl-2-hexenoate (A/10^7)	**0.001**	Sweet, fruity, juicy, rum, green, vegetal	1, 3, 4, 7
ethyl malate (A/10^7)	not available	Caramel, sugar, brown sugar, sweet, wine, fruity, herbal	1, 3, 4, 7
unidentifid terpene 1 (A/10^7)	not available	non available	non available
unidentifid terpene 2 (A/10^7)	not available	non available	non available
unidentified sesquiterpene 1 (A/10^7)	not available	non available	non available
unidentified sesquiterpene 2 (A/10^7)	not available	non available	non available
unidentified sesquiterpene 3 (A/10^7)	not available	non available	non available
unidentified sesquiterpene 4 (A/10^7)	not available	non available	non available

1 = Fruity; 2 = Floral; 3 = Green, Fresh; 4 = Sweet; 5 = Spicy; 6 = Fatty; 7 = Others

Regarding ethyl butanoate, MBT, CTR and MAD showed to have the highest 3-year average values, with MED and DMR having lower averages. There were some significant differences between the values in each vintage but all the concentrations were small. Overall, the concentration values were in the range of micrograms to tenths of micrograms per litre for each modality. The range of concentration of the study samples was similar to the range of the commercially obtained wine references.

As for ethyl hexanoate, the concentration values were also in the range of micrograms to tenths of micrograms per litre for each modality, and just a few significantly different averages. CTR showed smaller 3-year average value when compared with all other modalities, which have shown similar averages. The range of concentration of the studied samples was similar to the range of the commercially obtained wine references.

Concerning ethyl heptanoate, even though some significant differences, the 3-year averages were very closed, and the concentration values were in the range of micrograms grams per litre for each modality. The range of concentration of the studied samples was similar to the range of the commercially obtained wine references.

For ethyl octanoate, the average concentration of all modalities, for 2012 vintage samples, was higher than the remaining vintages, having significant differences between modalities in each vintage, the 3-year averages for MED and DMR were highest than for the other modalities. The concentration values were in the range of hundreds of micrograms to milligram per litre for each modality. The range of concentration of the studied samples was similar to the range of the commercially obtained wine references.

In relation to ethyl decanoate, despite the significant differences within each vintage, the 3-year average values were approximated and only DMR average was different and was the highest average of the group. The values for 2011 were smaller than for the remaining vintages. The concentration values were in the range of hundreds of micrograms to milligram per litre for each modality. The range of concentration of the studied samples was not similar to the range of the commercially obtained wine references, which were substantially higher.

Regarding ethyl dodecanoate, the 3-year average values were approximate, although the significant differences in each vintage. The values for 2011 were smaller than the other vintages. The concentration values were in the range of hundreds of micrograms per litre for each modality. The range of concentration of the study samples was not similar to the range of the commercially obtained wine references, which were substantially higher.

Table 38 - Ester content of the wines per modality, for 2011, 2012 and 2013 vintages. Average concentration values and standard deviation (between brackets).

Esters	Modality	2011 Averages	2012 Averages	2013 Averages	3-Vintage Average	Wine	Averages
ethyl butanoate (mg/l)	CTR	0.014 (0.001) c	0.015 (0.001) c	0.003 (0.001) a	0.011	2013DMR30	0.007 (0.000)
	MAD	0.003 (0.000) a	0.021 (0.003) d	0.007 (0.001) a	0.010	2013BIO	0.009 (0.001)
	MED	0.002 (0.001) a	0.012 (0.001) b	0.010 (0.001) a	0.008	2011M	0.005 (0.001)
	MBT	0.002 (0.001) a	0.013 (0.000) bc	0.024 (0.023) a	0.013	2012M	0.019 (0.004)
	DMR	0.007 (0.001) b	0.003 (0.000) a	0.012 (0.001) a	0.007	2008MM	0.016 (0.002)
	Average	0.006	0.013	0.011		2005PR	0.008 (0.001)
ethyl hexanoate (mg/l)	CTR	0.011 (0.001) a	0.019 (0.002) a	0.009 (0.001) a	0.013	2013DMR30	0.010 (0.000)
	MAD	0.013 (0.002) a	0.051 (0.006) c	0.013 (0.001) b	0.026	2013BIO	0.013 (0.001)
	MED	0.012 (0.003) a	0.039 (0.006) b	0.013 (0.001) b	0.021	2011M	0.022 (0.004)
	MBT	0.011 (0.001) a	0.037 (0.002) b	0.012 (0.001) b	0.020	2012M	0.040 (0.004)
	DMR	0.017 (0.002) b	0.035 (0.004) b	0.012 (0.000) b	0.021	2008MM	0.034 (0.002)
	Average	0.013	0.036	0.012		2005PR	0.037 (0.001)
ethyl heptanoate (mg/l)	CTR	0.003 (0.000) a	0.007 (0.001) c	0.003 (0.001) ab	0.005	2013DMR30	0.022 (0.002)
	MAD	0.003 (0.001) a	0.004 (0.001) ab	0.006 (0.001) c	0.004	2013BIO	0.004 (0.001)
	MED	0.002 (0.001) a	0.004 (0.001) ab	0.004 (0.000) b	0.003	2011M	0.004 (0.001)
	MBT	0.008 (0.000) b	0.005 (0.001) b	0.003 (0.000) a	0.005	2012M	0.002 (0.000)
	DMR	0.009 (0.001) c	0.003 (0.001) a	0.007 (0.001) c	0.006	2008MM	0.005 (0.001)
	Average	0.005	0.004	0.005		2005PR	0.004 (0.001)
ethyl octanoate (mg/l)	CTR	0.490 (0.023) c	1.170 (0.056) ab	0.557 (0.016) a	0.739	2013DMR30	0.542 (0.005)
	MAD	0.216 (0.007) a	1.435 (0.275) b	0.727 (0.007) b	0.793	2013BIO	0.756 (0.034)
	MED	0.274 (0.011) b	1.905 (0.012) c	0.888 (0.024) c	1.022	2011M	0.866 (0.037)
	MBT	0.270 (0.008) b	1.029 (0.060) a	0.742 (0.033) b	0.680	2012M	1.805 (0.254)
	DMR	0.466 (0.045) c	1.448 (0.247) b	0.857 (0.005) c	0.924	2008MM	1.619 (0.084)
	Average	0.343	1.397	0.754		2005PR	1.550 (0.202)
ethyl decanoate (mg/l)	CTR	0.403 (0.029) c	0.314 (0.000) a	0.393 (0.005) a	0.370	2013DMR30	0.818 (0.029)
	MAD	0.110 (0.011) a	0.312 (0.044) a	0.518 (0.027) b	0.313	2013BIO	0.668 (0.022)
	MED	0.098 (0.022) a	0.335 (0.014) a	0.595 (0.010) b	0.343	2011M	1.064 (0.078)
	MBT	0.186 (0.064) b	0.274 (0.015) a	0.586 (0.013) b	0.349	2012M	1.986 (0.130)
	DMR	0.213 (0.017) b	1.174 (0.339) b	0.924 (0.109) c	0.770	2008MM	1.398 (0.096)
	Average	0.202	0.482	0.603		2005PR	0.820 (0.017)
ethyl dodecanoate (mg/l)	CTR	0.099 (0.006) b	0.067 (0.005) a	0.112 (0.016) a	0.093	2013DMR30	0.427 (0.043)
	MAD	0.052 (0.001) a	0.094 (0.023) a	0.261 (0.013) c	0.136	2013BIO	0.439 (0.001)
	MED	0.040 (0.004) a	0.123 (0.012) a	0.390 (0.035) d	0.184	2011M	0.109 (0.006)
	MBT	0.108 (0.033) b	0.070 (0.014) a	0.183 (0.012) b	0.120	2012M	0.344 (0.063)
	DMR	0.044 (0.007) a	0.333 (0.063) b	0.182 (0.045) b	0.186	2008MM	0.153 (0.004)
	Average	0.069	0.137	0.226		2005PR	0.278 (0.211)
isoamyl acetate (mg/l)	CTR	0.059 (0.008) a	0.071 (0.006) a	0.149 (0.023) a	0.093	2013DMR30	0.168 (0.004)
	MAD	1.118 (0.187) c	0.150 (0.020) bc	0.330 (0.004) c	0.533	2013BIO	0.183 (0.009)
	MED	0.670 (0.161) b	0.166 (0.028) c	0.239 (0.003) b	0.359	2011M	0.079 (0.018)
	MBT	0.210 (0.016) a	0.137 (0.010) bc	0.165 (0.009) a	0.171	2012M	0.167 (0.010)
	DMR	0.104 (0.013) a	0.125 (0.014) b	0.144 (0.016) a	0.125	2008MM	0.068 (0.004)
	Average	0.432	0.130	0.205		2005PR	0.036 (0.002)
phenylethyl acetate (mg/l)	CTR	0.075 (0.006) b	0.050 (0.001) b	0.137 (0.001) d	0.087	2013DMR30	0.047 (0.001)
	MAD	0.735 (0.031) e	0.044 (0.001) ab	0.234 (0.001) e	0.337	2013BIO	0.087 (0.001)
	MED	0.404 (0.006) d	0.066 (0.007) c	0.111 (0.003) c	0.194	2011M	0.032 (0.002)
	MBT	0.102 (0.004) c	0.100 (0.010) d	0.096 (0.012) b	0.099	2012M	0.060 (0.001)
	DMR	0.045 (0.006) a	0.038 (0.003) a	0.038 (0.006) a	0.040	2008MM	0.028 (0.006)
	Average	0.272	0.059	0.123		2005PR	0.055 (0.014)
Total esters (mg/l)	CTR	1.153	1.713	1.364	1.410	2013DMR30	2.041
	MAD	2.249	2.110	2.097	2.152	2013BIO	2.160
	MED	1.503	2.649	2.251	2.134	2011M	2.181
	MBT	0.898	1.664	1.812	1.458	2012M	4.422
	DMR	0.905	3.158	2.175	2.079	2008MM	3.320
	Average	1.341	2.259	1.940	1.847	2005PR	2.788

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*'). Different letters indicate significantly different averages, according to Duncan test $p<0.05$.

Concerning *iso*amyl acetate, the concentration values were in the range of hundreds of micrograms per litre for each modality. The values for 2011 were higher than for the other vintages. MAD and MED showed the highest 3-year average. The range of

concentration of the studied samples was not similar to the range of the commercially obtained wine references, which were substantially lower.

For phenylethyl acetate, the concentration values were in the range of hundreds of micrograms per litre for each modality. MAD and MED showed the highest 3-year average, and the values for 2011 were higher than the remaining vintages. The range of concentration of the study samples was not similar to the range of the commercially obtained wine references, which were substantially lower.

Overall, there were four compounds with concentrations above all others - ethyl octanoate and ethyl decanoate (both ethyl esters) and isoamyl acetate and phenylethyl acetate (both acetates). These compounds displayed substantial vintage differences, with 2011 more favourable for acetate formation and ethyl esters were present in higher concentrations for 2012 and 2013. Using the total ester concentration and the 3-year average for each modality, it was observed that MAD and MED had the highest concentrations of esters from all modalities. This behaviour was very clear in 2011 and 2012 vintages, not so pronounced in 2013. CTR and MBT showed to have the lowest ester concentration and DMR displayed intermediate values. The range of total concentration of the study samples was lower when compared to the range of the commercially obtained wine references. These differences could be related with the ageing of commercial wines and the different fermentation conditions.

As for the remaining volatile esters (Table 39), only chromatographic areas were available (the concentrations were not calculated), the values for ethyl malate for 2011 were higher than for the other vintages. MBT and DMR showed the highest 3-year average and MED the lowest average. The range of areas of the studied samples was not similar to the range of the commercially obtained wine references, which were higher.

Regarding ethyl 2-methyl-butanoate, the areas of 2013 vintage were smaller than for the other vintages. MBT showed the highest 3-year average and CTR the lowest average. The range of areas of the studied samples was not similar to the range of the commercially obtained wine references, which were smaller.

Concerning ethyl 3-methyl-butanoate, the areas of 2011 vintage were higher than those for the other vintages. MAD and MED showed the highest 3-year average and CTR the lowest average. The range of areas of the study samples was not similar to the range of the commercially obtained wine references, which were smaller.

As to ethyl *trans*-4-decenoate, the areas of 2011 vintage were smaller than those for the remaining vintages. The 3-year average values were similar. The range of areas of

the study samples was similar to the range of the commercially obtained wine references.

About *iso*amyl hexanoate, the areas of 2012 vintage were higher than those for the other vintages. The 3-year average values were similar. The range of areas of the studied samples was similar to the range of the commercially obtained wine references.

Concerning ethyl 2-hexenoate, MBT showed the highest value and the range of areas of the study samples was similar to the range of the commercially obtained wine references.

Table 39 - Esters content of the wines per modality, for 2011, 2012 and 2013 vintages. Average chromatographic area values and standard deviation (between brackets).

Esters	Modality	2011 Averages	2012 Averages	2013 Averages	3-Vintage Average	Wine	Averages
ethyl malate (A/10^7)	CTR	0.046 (0.002) b	0.015 (0.003) a	n.d.	0.030	2013DMR30	n.d.
	MAD	0.046 (0.002) b	0.022 (0.002) ab	n.d.	0.034	2013BIO	n.d.
	MED	0.033 (0.006) a	0.006 (0.001) a	n.d.	0.020	2011M	0.028 (0.006)
	MBT	0.123 (0.008) d	0.015 (0.001) a	n.d.	0.069	2012M	0.005 (0.000)
	DMR	0.084 (0.002) c	0.038 (0.025) b	n.d.	0.061	2008MM	0.016 (0.001)
	Average	0.066	0.019	n.a.		2005PR	0.020 (0.002)
ethyl-2-methyl-butanoate (A/10^7)	CTR	0.013 (0.002) a	0.008 (0.001) a	n.d. a	0.010	2013DMR30	n.d.
	MAD	0.554 (0.023) d	0.013 (0.002) b	0.003 (0.000) c	0.190	2013BIO	n.d.
	MED	0.290 (0.072) c	0.028 (0.001) c	0.002 (0.000) b	0.107	2011M	0.023 (0.001)
	MBT	0.114 (0.012) b	0.012 (0.003) b	0.002 (0.000) b	0.042	2012M	0.007 (0.001)
	DMR	0.028 (0.001) a	0.032 (0.003) d	n.d. a	0.030	2008MM	0.024 (0.002)
	Average	0.200	0.019	0.002		2005PR	0.057 (0.005)
ethyl-3-methyl-butanoate (A/10^7)	CTR	0.023 (0.003) a	0.012 (0.001) a	0.023 (0.003) c	0.019	2013DMR30	0.009 (0.000)
	MAD	0.355 (0.026) d	0.015 (0.002) a	0.001 (0.000) a	0.123	2013BIO	0.002 (0.000)
	MED	0.263 (0.053) c	0.027 (0.002) b	0.002 (0.001) a	0.097	2011M	0.024 (0.001)
	MBT	0.104 (0.018) b	0.014 (0.002) a	0.010 (0.002) b	0.043	2012M	0.007 (0.001)
	DMR	0.023 (0.004) a	0.033 (0.002) c	0.012 (0.001) b	0.023	2008MM	0.022 (0.002)
	Average	0.154	0.020	0.009		2005PR	0.055 (0.004)
ethyl-*trans*-4-decenoate (A/10^7)	CTR	0.061 (0.006) c	0.208 (0.008) b	0.156 (0.006) b	0.142	2013DMR30	0.156 (0.002)
	MAD	0.033 (0.014) b	0.224 (0.020) b	0.205 (0.003) a	0.154	2013BIO	0.069 (0.013)
	MED	0.014 (0.002) a	0.128 (0.018) a	0.193 (0.007) c	0.112	2011M	0.148 (0.007)
	MBT	0.029 (0.005) b	0.159 (0.009) a	0.135 (0.006) cd	0.108	2012M	0.171 (0.014)
	DMR	0.138 (0.004) d	0.203 (0.028) b	0.069 (0.008) d	0.137	2008MM	0.219 (0.018)
	Average	0.055	0.184	0.152		2005PR	0.103 (0.003)
isoamyl hexanoate (A/10^7)	CTR	0.013 (0.001) b	0.012 (0.000) b	0.007 (0.001) a	0.011	2013DMR30	0.005 (0.001)
	MAD	n.d.	0.018 (0.004) c	0.011 (0.001) b	0.014	2013BIO	0.015 (0.001)
	MED	n.d.	0.034 (0.004) d	0.012 (0.000) b	0.023	2011M	0.004 (0.000)
	MBT	n.d.	0.014 (0.001) bc	0.011 (0.001) b	0.013	2012M	0.016 (0.003)
	DMR	n.d.	0.004 (0.000) a	0.007 (0.000) a	0.005	2008MM	0.004 (0.000)
	Average	0.013	0.016	0.010		2005PR	0.003 (0.001)
ethyl-2-hexenoate (A/10^7)	CTR	n.d.	n.d.	0.002 (0.000) c	0.002	2013DMR30	0.002 (0.000)
	MAD	n.d.	n.d.	0.002 (0.000) a	0.002	2013BIO	0.004 (0.000)
	MED	n.d.	n.d.	0.002 (0.000) b	0.002	2011M	n.d.
	MBT	n.d.	n.d.	0.005 (0.000) d	0.005	2012M	n.d.
	DMR	n.d.	n.d.	0.002 (0.000) c	0.002	2008MM	n.d.
	Average	n.a.	n.a.	0.003		2005PR	n.d.

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*'). Different letters indicate significantly different averages, according to Duncan test $p<0.05$.

Table 40 shows terpene concentrations in the wines from the study and for several commercial wine used as references.

Despite the significant differences observed within each vintage, for β-linalool, the 3-year average values were approximate and only DMR average was lower than the average of the group. The values for 2013 were higher than for wines from the other vintages. The concentration values were in the range of micrograms per litre for each modality. The range of concentration of the studied samples was similar to the range of the commercial wines, even though the average from BIO modality was higher than for the other samples.

Limonene was detected and quantified in only one vintage, 2013. The values were all significantly different, with MAD, CTR and MED obtaining the highest values and DMR showing a concentration 5-fold lower than this group. The concentration values were in the range of micrograms per litre for each modality. The range of concentration of the study samples was similar to the range of the commercial wine references.

Geranyl acetone was only detected and quantified in 2013 vintage. The values were all significantly different, with CTR being the highest value and the remaining modalities showing lower concentrations than CTR. The concentration values were in the range of micrograms per litre for each modality. The range of concentration of the studied samples was similar to the range of the commercial wines.

For nerolidol, the concentration values were in the range of decimal micrograms per litre for each modality. MAD and MBT showed the highest 3-year average, and the values for 2013 were smaller than for the other vintages. The range of concentration of the studied samples was similar to the range of the commercially obtained wine references.

Neryl acetate was detected and quantified in wines from 2011 and 2012 vintages. DMR showed to have the lowest significant concentration for both vintages, and the other modalities displayed similar values for both vintages. MAD and MED had the highest concentrations, with CTR with approximate values, and MBT lower than these three. The concentration values were in the range of micrograms per litre for each modality. The range of concentration of the studied samples was similar to the range of the commercial wines.

The values for α-terpineol, for 2011 and 2012 vintages, showed significant differences between the modalities, with DMR showing the lowest values. CTR, MAD and MED showed the highest 3-year average. The range of concentration of the studied samples was similar to the range of the commercial wines.

Table 40 - Terpene and terpene derivatives content of the wines per modality, for 2011, 2012 and 2013 vintages. Average concentration values and standard deviation (between brackets).

Terpenes	Modality	2011 Averages	2012 Averages	2013 Averages	3-Vintage Average	Wine	Averages
β - linalool (µg/l)	CTR	0.005 (0.001) d	0.005 (0.001) b	0.330 (0.046) c	0.114	2013DMR30	0.083 (0.003)
	MAD	0.001 (0.000) a	0.012 (0.000) e	0.377 (0.008) cd	0.130	2013BIO	0.318 (0.007)
	MED	0.001 (0.000) a	0.007 (0.000) c	0.400 (0.020) d	0.136	2011M	0.006 (0.000)
	MBT	0.002 (0.000) b	0.010 (0.001) d	0.264 (0.029) b	0.092	2012M	0.012 (0.000)
	DMR	0.003 (0.000) c	0.002 (0.000) a	0.076 (0.003) a	0.027	2008MM	0.010 (0.001)
	Average	0.002	0.007	0.289		2005PR	n.d.
limonene (µg/l)	CTR	n.d.	n.d.	0.604 (0.017) d	0.604	2013DMR30	0.211 (0.004)
	MAD	n.d.	n.d.	0.662 (0.037) e	0.662	2013BIO	0.486 (0.009)
	MED	n.d.	n.d.	0.547 (0.002) c	0.547	2011M	n.d.
	MBT	n.d.	n.d.	0.461 (0.036) b	0.461	2012M	n.d.
	DMR	n.d.	n.d.	0.177 (0.011) a	0.177	2008MM	n.d.
	Average	n.a.	n.a.	0.490		2005PR	n.d.
geranyl acetone (µg/l)	CTR	n.d.	n.d.	0.332 (0.024) c	0.332	2013DMR30	0.045 (0.001)
	MAD	n.d.	n.d.	0.086 (0.012) b	0.086	2013BIO	0.117 (0.016)
	MED	n.d.	n.d.	0.091 (0.001) b	0.091	2011M	n.d.
	MBT	n.d.	n.d.	0.086 (0.001) b	0.086	2012M	n.d.
	DMR	n.d.	n.d.	0.039 (0.008) a	0.039	2008MM	n.d.
	Average	n.a.	n.a.	0.127		2005PR	n.d.
nerolidol (µg/l)	CTR	0.005 (0.000) a	0.003 (0.000) a	0.003 (0.001) c	0.004	2013DMR30	0.001 (0.000)
	MAD	0.007 (0.002) a	0.056 (0.006) d	0.003 (0.000) bc	0.022	2013BIO	0.002 (0.000)
	MED	0.004 (0.001) a	0.041 (0.007) c	0.004 (0.000) d	0.017	2011M	0.029 (0.003)
	MBT	0.031 (0.002) c	0.076 (0.005) e	0.002 (0.001) b	0.037	2012M	0.472 (0.092)
	DMR	0.017 (0.005) b	0.014 (0.004) b	0.001 (0.001) a	0.011	2008MM	0.030 (0.003)
	Average	0.013	0.038	0.003		2005PR	n.d.
neryl acetate (µg/l)	CTR	0.026 (0.0033) c	0.015 (0.0023) b	n.d.	0.021	2013DMR30	n.d.
	MAD	0.028 (0.0035) c	0.015 (0.0019) b	n.d.	0.021	2013BIO	n.d.
	MED	0.030 (0.0028) c	0.015 (0.0036) b	n.d.	0.023	2011M	0.005 (0.0009)
	MBT	0.014 (0.0012) b	0.016 (0.0025) b	n.d.	0.015	2012M	0.017 (0.0014)
	DMR	0.005 (0.0007) a	n.d.	n.d.	0.003	2008MM	0.015 (0.0030)
	Average	0.021	0.012	n.a.		2005PR	0.015 (0.0014)
α-terpineol (µg/l)	CTR	0.010 (0.0000) d	0.007 (0.0006) b	0.005 (0.0000) b	0.007	2013DMR30	n.d.
	MAD	0.005 (0.0010) b	0.010 (0.0000) d	n.d.	0.005	2013BIO	n.d.
	MED	0.007 (0.0006) c	0.008 (0.0012) bc	n.d.	0.005	2011M	0.002 (0.0000)
	MBT	0.003 (0.0012) a	0.009 (0.0006) c	n.d.	0.004	2012M	0.005 (0.0006)
	DMR	0.003 (0.0006) a	n.d.	n.d.	0.001	2008MM	0.008 (0.0010)
	Average	0.006	0.007	0.001		2005PR	0.004 (0.0006)
β-*cis*-terpineol (µg/l)	CTR	0.009 (0.0001) c	0.005 (0.0001) b	0.005 (0.0003) b	0.006	2013DMR30	0.005 (0.0004)
	MAD	0.010 (0.0003) d	n.d.	0.006 (0.0006) c	0.005	2013BIO	0.005 (0.0001)
	MED	0.003 (0.0003) a	n.d.	0.005 (0.0004) ab	0.003	2011M	0.002 (0.0002)
	MBT	0.005 (0.0006) b	n.d.	0.005 (0.0001) bc	0.003	2012M	0.012 (0.0002)
	DMR	0.003 (0.0002) a	0.029 (0.0006) c	0.004 (0.0003) a	0.012	2008MM	n.d.
	Average	0.006	0.007	0.005		2005PR	n.d.
terpinolene (µg/l)	CTR	n.d.	n.d.	0.111 (0.0006) d	0.111	2013DMR30	0.078 (0.0015)
	MAD	n.d.	n.d.	0.116 (0.0010) e	0.116	2013BIO	0.097 (0.0000)
	MED	n.d.	n.d.	0.107 (0.0010) c	0.107	2011M	n.d.
	MBT	n.d.	n.d.	0.095 (0.0010) b	0.095	2012M	n.d.
	DMR	n.d.	n.d.	0.067 (0.0010) a	0.067	2008MM	n.d.
	Average	n.a.	n.a.	0.099		2005PR	n.d.
Total terpenes (µg/l)	CTR	0.055	0.035	1.391	0.494	2013DMR30	0.424
	MAD	0.051	0.093	1.249	0.464	2013BIO	1.025
	MED	0.045	0.071	1.153	0.423	2011M	0.044
	MBT	0.055	0.111	0.914	0.360	2012M	0.517
	DMR	0.032	0.045	0.364	0.147	2008MM	0.063
	Average	0.048	0.071	1.014		2005PR	0.019

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*'). Different letters indicate significantly different averages, according to Duncan test $p<0.05$.

Concerning β-*cis*-terpineol, the concentration values were in the range of micrograms per litre for each modality. The values for all vintages were similar, having some significant differences within each vintage. CTR and MAD presented the highest 3-year average. The range of concentration of the studied samples was similar to the range of the commercial wines.

Terpinolene was detected and quantified in only one vintage, 2013. The values were all significantly different, with MAD, CTR and MBT having the highest values. DMR displayed the lowest value for all modalities. The concentration values were in the range of micrograms per litre for each modality. The range of concentration of the studied samples was similar to the range of the commercial wines.

Globally, and using the total terpenes 3-year average concentration, it was observed that 2011 and 2012 vintages had lower total terpene concentration than 2013. The range of total concentration of the study samples was similar to the range of the commercially obtained wine references. The only exception was 2013 BIO that displayed a concentration twice higher than average values.

Table 41 displays some terpenes content of the wines per modality, displaying average chromatographic areas.

cis-β-Farnesene was identified only in wines from 2013 vintage, and the values showed significant differences. Wines from DMR modality was obtained the lowest value and MED the highest, with CTR and MAD having an intermediated value. The obtained chromatographic areas were similar to the range of the commercial wines.

trans-α-Bisabolene was only identified in the 2013 wine vintage. The obtained chromatographic areas for all modalities were similar, except for CTR that displayed a significantly higher area. The values were similar to the range of the commercially obtained wine references.

trans-Nerolidol was only identified in the 2013 vintage wines. The area values showed significant differences. DMR was the lowest value and MED, MAD and MBT were in an intermediate level. CTR presented the highest chromatographic area. The obtained areas were similar to the range of the commercial wines.

Table 42 displays other than ester or terpene compounds concentrations in the wines from the study and several commercially obtained wine references (only for comparison) and Table 43 shows analytical chromatographic areas for the wines from the study and references. Even though the chromatographic areas do not provide information about the concentration of these compounds in each sample, it could still provide some information about how each modality influences grape and/or wine quality in each vintage.

Table 41 - Terpenes content of the wines per modality, for 2011, 2012 and 2013 vintages. Average chromatographic area values and standard deviation (between brackets).

Terpenes	Modality	2011 Averages	2012 Averages	2013 Averages	3-Vintage Average	Wine	Averages
S-α-pinene (A/10^7)	CTR	n.d.	n.d.	0.000 (0.000) a	0.000	2013DMR30	0.013 (0.001)
	MAD	n.d.	n.d.	0.002 (0.001) ab	0.002	2013BIO	0.007 (0.001)
	MED	n.d.	n.d.	0.004 (0.001) c	0.004	2011M	n.d.
	MBT	n.d.	n.d.	0.002 (0.000) bc	0.002	2012M	n.d.
	DMR	n.d.	n.d.	0.012 (0.002) d	0.012	2008MM	n.d.
	Average	n.a.	n.a.	0.004		2005PR	n.d.
unidentifid terpene 1 (A/10^7)	CTR	n.d.	n.d.	0.026 (0.010) a	0.026	2013DMR30	0.070 (0.002)
	MAD	n.d.	n.d.	0.053 (0.004) bc	0.053	2013BIO	0.052 (0.007)
	MED	n.d.	n.d.	0.058 (0.003) c	0.058	2011M	n.d.
	MBT	n.d.	n.d.	0.048 (0.000) b	0.048	2012M	n.d.
	DMR	n.d.	n.d.	0.080 (0.004) d	0.080	2008MM	n.d.
	Average	n.a.	n.a.	0.053		2005PR	n.d.
unidentifid terpene 2 (A/10^7)	CTR	n.d.	n.d.	0.028 (0.005) a	0.028	2013DMR30	0.034 (0.001)
	MAD	n.d.	n.d.	0.034 (0.000) c	0.034	2013BIO	0.023 (0.001)
	MED	n.d.	n.d.	0.028 (0.001) a	0.028	2011M	n.d.
	MBT	n.d.	n.d.	0.029 (0.001) ab	0.029	2012M	n.d.
	DMR	n.d.	n.d.	0.033 (0.002) bc	0.033	2008MM	n.d.
	Average	n.a.	n.a.	0.030		2005PR	n.d.
cis-β-farnesene (A/10^7)	CTR	n.d.	n.d.	2.377 (0.176) b	2.377	2013DMR30	0.885 (0.067)
	MAD	n.d.	n.d.	2.224 (0.356) b	2.224	2013BIO	1.608 (0.225)
	MED	n.d.	n.d.	3.407 (0.205) c	3.407	2011M	n.d.
	MBT	n.d.	n.d.	1.898 (0.301) b	1.898	2012M	n.d.
	DMR	n.d.	n.d.	0.641 (0.245) a	0.641	2008MM	n.d.
	Average	n.a.	n.a.	2.109		2005PR	n.d.
unidentified sesquiterpene 1 (A/10^7)	CTR	n.d.	n.d.	2.959 (0.162) b	2.959	2013DMR30	2.270 (0.366)
	MAD	n.d.	n.d.	1.922 (0.118) a	1.922	2013BIO	1.280 (0.121)
	MED	n.d.	n.d.	1.610 (0.112) a	1.610	2011M	n.d.
	MBT	n.d.	n.d.	1.599 (0.148) a	1.599	2012M	n.d.
	DMR	n.d.	n.d.	1.795 (0.444) a	1.795	2008MM	n.d.
	Average	n.a.	n.a.	1.977		2005PR	n.d.
unidentified sesquiterpene 2 (A/10^7)	CTR	n.d.	n.d.	0.982 (0.108) b	0.982	2013DMR30	0.470 (0.014)
	MAD	n.d.	n.d.	0.997 (0.182) b	0.997	2013BIO	0.632 (0.001)
	MED	n.d.	n.d.	1.256 (0.149) c	1.256	2011M	n.d.
	MBT	n.d.	n.d.	0.806 (0.105) b	0.806	2012M	n.d.
	DMR	n.d.	n.d.	0.408 (0.091) a	0.408	2008MM	n.d.
	Average	n.a.	n.a.	0.890		2005PR	n.d.
unidentified sesquiterpene 3 (A/10^7)	CTR	n.d.	n.d.	0.386 (0.008) a	0.386	2013DMR30	3.848 (1.851)
	MAD	n.d.	n.d.	0.960 (0.555) a	0.960	2013BIO	0.948 (0.310)
	MED	n.d.	n.d.	0.318 (0.005) a	0.318	2011M	n.d.
	MBT	n.d.	n.d.	1.161 (0.590) a	1.161	2012M	n.d.
	DMR	n.d.	n.d.	9.581 (3.877) b	9.581	2008MM	n.d.
	Average	n.a.	n.a.	2.481		2005PR	n.d.
unidentified sesquiterpene 4 (A/10^7)	CTR	n.d.	n.d.	5.231 (0.059) c	5.231	2013DMR30	0.699 (0.004)
	MAD	n.d.	n.d.	4.633 (0.449) bc	4.633	2013BIO	3.547 (0.028)
	MED	n.d.	n.d.	9.307 (0.680) d	9.307	2011M	n.d.
	MBT	n.d.	n.d.	3.927 (0.607) b	3.927	2012M	n.d.
	DMR	n.d.	n.d.	0.899 (0.110) a	0.899	2008MM	n.d.
	Average	n.a.	n.a.	4.799		2005PR	n.d.
trans-α-bisabolene (A/10^7)	CTR	n.d.	n.d.	19.594 (5.407) b	19.594	2013DMR30	0.251 (0.009)
	MAD	n.d.	n.d.	0.472 (0.050) a	0.472	2013BIO	0.219 (0.042)
	MED	n.d.	n.d.	0.525 (0.100) a	0.525	2011M	n.d.
	MBT	n.d.	n.d.	0.393 (0.090) a	0.393	2012M	n.d.
	DMR	n.d.	n.d.	0.258 (0.090) a	0.258	2008MM	n.d.
	Average	n.a.	n.a.	4.248		2005PR	n.d.
trans-nerolidol (A/10^7)	CTR	n.d.	n.d.	172.624 (2.634) d	172.6	2013DMR30	0.662 (0.035)
	MAD	n.d.	n.d.	4.561 (0.457) b	4.561	2013BIO	3.554 (0.016)
	MED	n.d.	n.d.	9.404 (0.762) c	9.404	2011M	n.d.
	MBT	n.d.	n.d.	3.936 (0.601) b	3.936	2012M	n.d.
	DMR	n.d.	n.d.	0.914 (0.034) a	0.914	2008MM	n.d.
	Average	n.a.	n.a.	38.288		2005PR	n.d.

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*'). Different letters indicate significantly different averages, according to Duncan test $p<0.05$.

Table 42 - Several classes of compounds content of the wines per modality, for 2011, 2012 and 2013 vintages. Average concentration values and standard deviation (between brackets).

	Modality	2011 Averages	2012 Averages	2013 Averages	3-Vintage Average	Wine	Averages
phenylethyl alcohol (mg/l)	CTR	11.273 (0.483) d	6.888 (0.028) b	7.295 (0.030) b	8.485	2013DMR30	3.312 (0.090)
	MAD	4.724 (0.541) b	7.966 (0.347) bc	7.170 (0.280) b	6.620	2013BIO	8.093 (0.133)
	MED	3.660 (0.100) a	6.970 (1.396) b	9.650 (0.100) d	6.760	2011M	5.953 (0.639)
	MBT	5.166 (0.238) b	8.933 (0.151) c	8.493 (0.088) c	7.531	2012M	7.311 (0.196)
	DMR	5.984 (0.135) c	3.801 (0.553) a	3.840 (0.161) a	4.541	2008MM	5.625 (0.421)
	Average	6.161	6.911	7.289		2005PR	5.550 (0.173)
diethyl succianate (mg/l)	CTR	3.749 (0.145) c	2.982 (0.021) d	1.375 (0.024) d	2.702	2013DMR30	0.423 (0.013)
	MAD	0.541 (0.034) a	1.433 (0.091) b	0.567 (0.015) b	0.847	2013BIO	4.330 (0.050)
	MED	0.640 (0.060) a	1.026 (0.113) a	2.408 (0.036) e	1.358	2011M	8.141 (0.218)
	MBT	2.401 (0.060) b	1.043 (0.019) a	0.675 (0.010) c	1.373	2012M	2.227 (0.105)
	DMR	3.704 (0.029) c	1.662 (0.197) b	0.389 (0.001) a	1.918	2008MM	7.636 (0.347)
	Average	2.207	1.629	1.083		2005PR	11.753 (0.415)
β-damascenone (µg/l)	CTR	n.d.	n.d.	0.012 (0.001) d	0.012	2013DMR30	0.003 (0.000)
	MAD	n.d.	n.d.	0.014 (0.001) e	0.014	2013BIO	0.010 (0.001)
	MED	n.d.	n.d.	0.011 (0.000) c	0.011	2011M	n.d.
	MBT	n.d.	n.d.	0.009 (0.001) b	0.009	2012M	n.d.
	DMR	n.d.	n.d.	0.002 (0.001) a	0.002	2008MM	n.d.
	Average	n.a.	n.a.	0.010		2005PR	n.d.

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*'). Different letters indicate significantly different averages, according to Duncan test $p<0.05$.

Concerning phenylethyl alcohol, the concentration values were in the range of several milligrams per litre for each modality. The average values for all vintages were similar. CTR and MBT showed the highest 3-year average and DMR the lowest average. The range of concentration of the study samples was similar to the range of the commercially obtained wine references.

Diethyl succinate concentrations range of hundreds of micrograms to milligrams per litre for each modality. The values for 2011 were higher than the remaining vintages. CTR and DMR showed the highest 3-year average and MAD the lowest average. The range of concentration of the study samples was not similar to the range of the commercially obtained wine references, which were substantially higher.

β-Damascenone was only identified and quantified in 2013 vintage. There were significant differences between the concentrations obtained, being highest concentration found in MAD, followed by CTR, MED and MBT, with DMR having the lowest concentration. The concentration values were in the range of micrograms per litre for each modality. The range of concentration of the studied samples was similar to the range of the commercial wines.

The chromatographic areas obtained for several classes of compounds are present in Table 43.

4-Ethylguaiacol was identified only in wines from 2013 vintage, and the chromatographic area values showed significant differences. DMR has had the lowest value and MED the highest, with MAD having an intermediated level. CTR and MBT displayed similar low values. The obtained areas were similar to the range of the

commercially obtained wine references. 4-Ethylguaiacol was identified in commercial samples only.

For benzyl alcohol, the values for vintages were similar and similar to the range obtained for the commercial wine references. MAD showed the highest 3-year average.

Table 43 - Several classes of compounds content of the wines per modality, for 2011, 2012 and 2013 vintages. Average chromatographic area values and standard deviation (between brackets).

Others	Modality	2011 Averages	2012 Averages	2013 Averages	3-Vintage Average	Wine	Averages
4-ethylguaiacol (A/10^7)	CTR	n.d.	n.d.	1.893 (0.062) a	1.893	2013DMR30	0.841 (0.026)
	MAD	n.d.	n.d.	11.732 (2.073) b	11.732	2013BIO	1.292 (0.199)
	MED	n.d.	n.d.	113.283 (6.369) c	113.3	2011M	n.d.
	MBT	n.d.	n.d.	3.774 (0.847) a	3.774	2012M	n.d.
	DMR	n.d.	n.d.	0.361 (0.068) a	0.361	2008MM	n.d.
	Average	n.a.	n.a.	26.21		2005PR	n.d.
4-ethylphenol (A/10^7)	CTR	n.d.	n.d.	n.d.	n.d.	2013DMR30	n.d.
	MAD	n.d.	n.d.	n.d.	n.d.	2013BIO	n.d.
	MED	n.d.	n.d.	n.d.	n.d.	2011M	n.d.
	MBT	n.d.	n.d.	n.d.	n.d.	2012M	0.024 (0.004)
	DMR	n.d.	n.d.	n.d.	n.d.	2008MM	0.000 (0.000)
	Average	n.a.	n.a.	n.a.	n.a.	2005PR	0.136 (0.020)
benzyl alcohol (A/10^7)	CTR	0.014 (0.003) a	0.011 (0.001) b	0.011 (0.001) a	0.012	2013DMR30	0.009 (0.001)
	MAD	0.020 (0.003) b	0.024 (0.002) c	0.062 (0.050) b	0.035	2013BIO	0.040 (0.001)
	MED	0.029 (0.002) c	0.024 (0.003) c	0.017 (0.001) a	0.023	2011M	0.016 (0.001)
	MBT	0.021 (0.002) b	0.021 (0.002) c	0.016 (0.001) a	0.020	2012M	0.032 (0.001)
	DMR	0.033 (0.002) c	0.004 (0.001) a	0.011 (0.001) a	0.016	2008MM	0.022 (0.001)
	Average	0.023	0.017	0.023		2005PR	0.031 (0.003)
TDN (A/10^7)	CTR	0.293 (0.013) b	0.032 (0.005) b	n.d.	0.163	2013DMR30	n.d.
	MAD	0.650 (0.032) d	0.037 (0.003) b	n.d.	0.343	2013BIO	n.d.
	MED	0.458 (0.082) c	0.049 (0.003) c	n.d.	0.254	2011M	0.046 (0.001)
	MBT	0.077 (0.004) a	0.031 (0.003) b	n.d.	0.054	2012M	0.025 (0.002)
	DMR	0.105 (0.006) a	0.021 (0.002) a	n.d.	0.063	2008MM	0.121 (0.008)
	Average	0.317	0.034	n.a.		2005PR	0.348 (0.035)
dihydro pseudoionone (A/10^7)	CTR	n.d.	n.d.	0.855 (0.008) d	0.855	2013DMR30	0.949 (0.016)
	MAD	n.d.	n.d.	0.944 (0.026) e	0.944	2013BIO	0.057 (0.021)
	MED	n.d.	n.d.	0.315 (0.007) b	0.315	2011M	n.d.
	MBT	n.d.	n.d.	0.244 (0.050) a	0.244	2012M	n.d.
	DMR	n.d.	n.d.	0.392 (0.022) c	0.392	2008MM	n.d.
	Average	n.a.	n.a.	0.550		2005PR	n.d.

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*'). Different letters indicate significantly different averages, according to Duncan test $p<0.05$.

TDN was detected in 2011 and 2012 vintages. The obtained values were significantly different, with MAD and MED being the highest values and MBT and DMR showing the lowest values of the group. The range of the chromatographic areas of the studied samples was similar to the range of the commercial wines.

Dihydro pseudoionone was only detected in wines from 2013 vintage. The chromatographic areas obtained were significantly different, with MAD, CTR having the highest values and MBT the lowest. The range of areas of the studied samples was similar to the range of the commercial wines.

6.2 Odour Activity Values (OAV). Baga varietal aroma compounds

Although the numerous volatile compounds present in a wine, only a restricted number of them contribute effectively to the aroma of the wine perception. The calculation of the odour activity (OAV) of each compound allows knowing the impact of this compound on the global aroma of a particular wine. The odour activity of a compound can be estimated by the ratio between the concentration of a volatile compound and its corresponding perception threshold, if described in literature [330]. Theoretically, when a value of odour activity for an individual compound is higher than the unity, this compound might be sensorially perceived and could have an impact on the overall aroma. One problem that might arise with the use of this odour activity estimation is that the odour thresholds for the studied variety, wine style or type or even for the same beverage are frequently not available in the literature. This leads to assumption that odour threshold available in literature is similar and equivalent, even if not estimated for the same beverage. Another problem is that each compound suffers influence from the matrix of the sample, not over the descriptors perceived but over its odour intensity [331]. Because of these problems, it was found in literature several suggestions to consider as valid sensorial contribution of compounds if OAV is about 0.2 (at least 20% of the threshold concentration) [332,333].

The odour activity values of the esters family compounds are displayed in Table 44. The estimation of OAV for each compound followed the threshold concentration obtained from literature for that specific compound.

From all the esters that odour activity estimation was possible (Table 37), ethyl hexanoate, ethyl octanoate, ethyl decanoate and isoamyl acetate, showed OAV values above the unity - these compounds should have impact on the aroma of Baga wines. Ethyl butanoate, ethyl heptanoate, ethyl dodecanoate and phenylethyl acetate displayed OAV values above 0.2 so their contribution on the aroma should not be neglected, even if the individual contribution could not be performed by individual tasters.

Another large group of compounds quantified were the terpenes. From all the terpenes that odour activity estimation was possible (Table 45), β-linalool and terpinolene showed OAV values below 1 but above 0.2, thus their contribution on the aroma should not be ignored. Given the numerous terpenes present in Baga wines, even if present in low concentrations, some synergic contributions for the global aroma between these compounds might occur.

Table 44 - Odour activity values (OAV) for esters of the wines per modality, for 2011, 2012 and 2013 vintages and for commercially obtained wine references. Average values.

		OAV			3 Year Average		
Esters	**Modality**	**2011**	**2012**	**2013**		**Wines**	**OAV**
ethyl butanoate	**CTR**	0.68	0.75	0.17	0.53	**2013DMR30**	0.35
	MAD	0.15	1.05	0.37	0.52	**2013BIO**	0.45
	MED	0.12	0.58	0.50	0.40	**2011M**	0.27
	MBT	0.12	0.65	1.20	0.66	**2012M**	0.97
	DMR	0.33	0.15	0.60	0.36	**2008MM**	0.82
	Average	0.28	0.64	0.57	n.a.	**2005PR**	0.42
ethyl hexanoate	**CTR**	0.81	1.33	0.64	0.93	**2013DMR30**	0.71
	MAD	0.93	3.62	0.93	1.83	**2013BIO**	0.93
	MED	0.86	2.79	0.95	1.53	**2011M**	1.57
	MBT	0.79	2.64	0.88	1.44	**2012M**	2.83
	DMR	1.19	2.48	0.86	1.51	**2008MM**	2.40
	Average	0.91	2.57	0.85	n.a.	**2005PR**	2.62
ethyl heptanoate	**CTR**	0.14	0.33	0.15	0.21	**2013DMR30**	1.00
	MAD	0.12	0.18	0.29	0.20	**2013BIO**	0.20
	MED	0.11	0.17	0.18	0.15	**2011M**	0.17
	MBT	0.36	0.21	0.14	0.24	**2012M**	0.09
	DMR	0.42	0.12	0.30	0.28	**2008MM**	0.21
	Average	0.23	0.20	0.21	n.a.	**2005PR**	0.18
ethyl octanoate	**CTR**	98.1	234.1	111.5	147.9	**2013DMR30**	108.4
	MAD	43.2	287.1	145.4	158.6	**2013BIO**	151.3
	MED	54.9	380.9	177.7	204.5	**2011M**	173.2
	MBT	54.0	205.9	148.3	136.1	**2012M**	360.9
	DMR	93.2	289.5	171.5	184.7	**2008MM**	323.8
	Average	68.7	279.5	150.9	n.a.	**2005PR**	310.0
ethyl decanoate	**CTR**	2.01	1.57	1.96	1.85	**2013DMR30**	4.09
	MAD	0.55	1.56	2.59	1.57	**2013BIO**	3.34
	MED	0.49	1.67	2.98	1.71	**2011M**	5.32
	MBT	0.93	1.37	2.93	1.74	**2012M**	9.93
	DMR	1.06	5.87	4.62	3.85	**2008MM**	6.99
	Average	1.01	2.41	3.02	n.a.	**2005PR**	4.10
ethyl dodecanoate	**CTR**	0.07	0.04	0.07	0.06	**2013DMR30**	0.28
	MAD	0.03	0.06	0.17	0.09	**2013BIO**	0.29
	MED	0.03	0.08	0.26	0.12	**2011M**	0.07
	MBT	0.07	0.05	0.12	0.08	**2012M**	0.23
	DMR	0.03	0.22	0.12	0.12	**2008MM**	0.10
	Average	0.05	0.09	0.15	n.a.	**2005PR**	0.19
isoamyl acetate	**CTR**	1.97	2.35	4.95	3.09	**2013DMR30**	5.60
	MAD	37.26	5.00	11.00	17.75	**2013BIO**	6.10
	MED	22.34	5.55	7.98	11.96	**2011M**	2.63
	MBT	6.99	4.56	5.51	5.69	**2012M**	5.55
	DMR	3.48	4.18	4.80	4.15	**2008MM**	2.26
	Average	14.41	4.33	6.85	n.a.	**2005PR**	1.20
phenylethyl acetate	**CTR**	0.30	0.20	0.55	0.35	**2013DMR30**	0.19
	MAD	2.94	0.18	0.93	1.35	**2013BIO**	0.35
	MED	1.62	0.26	0.44	0.77	**2011M**	0.13
	MBT	0.41	0.40	0.38	0.40	**2012M**	0.24
	DMR	0.18	0.15	0.15	0.16	**2008MM**	0.11
	Average	1.09	0.24	0.49	n.a.	**2005PR**	0.22

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*').

trans-Nerolidol showed OAV values above 1 and so, its contribution on the aroma should be perceptible. The value obtained for CTR in 2013 vintage was high, given some suspicion to the validity of this estimation.

Table 45 - Odour activity values (OAV) for Terpenes of the wines per modality, for 2011, 2012 and 2013 vintages and for commercially obtained wine references. Average values.

		OAV			3 Year		
Terpenes	**Modality**	**2011**	**2012**	**2013**	**Average**	**Wines**	**OAV**
β **- Linalol**	**CTR**	0.20	0.21	1.69	0.70	**2013DMR30**	0.00
	MAD	0.04	0.48	0.00	0.17	**2013BIO**	0.00
	MED	0.04	0.28	0.00	0.11	**2011M**	0.24
	MBT	0.08	0.41	0.00	0.16	**2012M**	0.48
	DMR	0.12	0.08	0.00	0.07	**2008MM**	0.41
	Average	0.10	0.29	0.34	n.a.	**2005PR**	0.00
Limonene	**CTR**	n.a.	n.a.	0.003	0.003	**2013DMR30**	0.001
	MAD	n.a.	n.a.	0.003	0.003	**2013BIO**	0.002
	MED	n.a.	n.a.	0.003	0.003	**2011M**	n.a.
	MBT	n.a.	n.a.	0.002	0.002	**2012M**	n.a.
	DMR	n.a.	n.a.	0.001	0.001	**2008MM**	n.a.
	Average	n.a.	n.a.	0.002	n.a.	**2005PR**	n.a.
Geranyl acetone	**CTR**	n.a.	n.a.	0.0018	0.0018	**2013DMR30**	0.0002
	MAD	n.a.	n.a.	0.0005	0.0005	**2013BIO**	0.0006
	MED	n.a.	n.a.	0.0005	0.0005	**2011M**	n.a.
	MBT	n.a.	n.a.	0.0005	0.0005	**2012M**	n.a.
	DMR	n.a.	n.a.	0.0002	0.0002	**2008MM**	n.a.
	Average	n.a.	n.a.	0.0007	n.a.	**2005PR**	n.a.
Nerolidol	**CTR**	0.007	0.004	0.005	0.005	**2013DMR30**	0.001
	MAD	0.010	0.080	0.004	0.031	**2013BIO**	0.003
	MED	0.006	0.059	0.006	0.024	**2011M**	0.042
	MBT	0.045	0.109	0.003	0.052	**2012M**	0.674
	DMR	0.024	0.020	0.002	0.015	**2008MM**	0.043
	Average	0.018	0.055	0.004	n.a.	**2005PR**	0.000
Neryl Acetate	**CTR**	0.00003	0.00002	n.a.	0.00002	**2013DMR30**	n.a.
	MAD	0.00003	0.00002	n.a.	0.00002	**2013BIO**	n.a.
	MED	0.00003	0.00002	n.a.	0.00003	**2011M**	0.00001
	MBT	0.00002	0.00002	n.a.	0.00002	**2012M**	0.00002
	DMR	0.00001	0.00000	n.a.	0.00000	**2008MM**	0.00002
	Average	0.00002	0.00001	n.a.	n.a.	**2005PR**	0.00002
α**-Terpineol**	**CTR**	0.025	0.017	0.013	0.018	**2013DMR30**	0.000
	MAD	0.013	0.025	0.000	0.013	**2013BIO**	0.000
	MED	0.017	0.019	0.000	0.012	**2011M**	0.005
	MBT	0.007	0.022	0.000	0.009	**2012M**	0.012
	DMR	0.008	0.000	0.000	0.003	**2008MM**	0.020
	Average	0.014	0.017	0.003	n.a.	**2005PR**	0.009
β**-cis-Terpineol**	**CTR**	0.022	0.012	0.012	0.015	**2013DMR30**	0.012
	MAD	0.026	0.000	0.014	0.013	**2013BIO**	0.012
	MED	0.007	0.000	0.012	0.006	**2011M**	0.005
	MBT	0.013	0.000	0.013	0.009	**2012M**	0.029
	DMR	0.008	0.073	0.010	0.030	**2008MM**	0.000
	Average	0.015	0.017	0.012	n.a.	**2005PR**	0.000
Terpinolene	**CTR**	n.a.	n.a.	0.56	0.56	**2013DMR30**	0.39
	MAD	n.a.	n.a.	0.58	0.58	**2013BIO**	0.49
	MED	n.a.	n.a.	0.54	0.54	**2011M**	n.a.
	MBT	n.a.	n.a.	0.48	0.48	**2012M**	n.a.
	DMR	n.a.	n.a.	0.34	0.34	**2008MM**	n.a.
	Average	n.a.	n.a.	0.50	n.a.	**2005PR**	n.a.

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*').

Phenylethyl alcohol exhibited OAV values below the unity but above 0.2, therefore its contribution on the aroma should not be neglected.

β-Damascenone showed OAV values below 1 but above 0.2, consequently its contribution on the aroma should be considered.

Table 46 - Odour activity values (OAV) for several classes of compounds of the wines per modality, for 2011, 2012 and 2013 vintages and for commercially obtained wine references. Average values.

		OAV			3 Year Average		
Others	**Modality**	**2011**	**2012**	**2013**		**Wines**	**OAV**
phenylethyl alcohol	**CTR**	1.13	0.69	0.73	0.85	**2013DMR30**	0.33
	MAD	0.47	0.80	0.72	0.66	**2013BIO**	0.81
	MED	0.37	0.70	0.97	0.68	**2011M**	0.60
	MBT	0.52	0.89	0.85	0.75	**2012M**	0.73
	DMR	0.60	0.38	0.38	0.45	**2008MM**	0.56
	Average	0.62	0.69	0.73	n.a.	**2005PR**	0.56
diethyl succianate	**CTR**	0.019	0.015	0.007	0.014	**2013DMR30**	0.002
	MAD	0.003	0.007	0.003	0.004	**2013BIO**	0.022
	MED	0.003	0.005	0.012	0.007	**2011M**	0.041
	MBT	0.012	0.005	0.003	0.007	**2012M**	0.011
	DMR	0.019	0.008	0.002	0.010	**2008MM**	0.038
	Average	0.011	0.008	0.005	n.a.	**2005PR**	0.059
β-damascenone	**CTR**	n.a.	n.a.	0.25	0.25	**2013DMR30**	0.06
	MAD	n.a.	n.a.	0.27	0.27	**2013BIO**	0.19
	MED	n.a.	n.a.	0.22	0.22	**2011M**	n.a.
	MBT	n.a.	n.a.	0.18	0.18	**2012M**	n.a.
	DMR	n.a.	n.a.	0.05	0.05	**2008MM**	n.a.
	Average	n.a.	n.a.	0.19	n.a.	**2005PR**	n.a.

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*').

Summarizing, ethyl hexanoate, ethyl octanoate, ethyl decanoate, isoamyl acetate and *trans*-nerolidol were present with odour activity above 1 and should have impact on the aroma of Baga wines. On the other hand, ethyl butanoate, ethyl heptanoate, ethyl dodecanoate, phenylethyl acetate, phenylethyl alcohol, β-linalool, terpinolene and β-damascenone exhibited OAV values above 0.2 (but below the unity) so their contribution on the aroma should not be neglected, even if the individual contribution could not be performed by individual tasters.

All other compounds showed OAV values below 0.2 or it was not possible to estimate odour activity because they do not have available odour threshold concentrations in literature, or the GC-MS method response coefficient for the compound was not available or not applicable.

The present OAV determination was measured which must be taken into account when Baga varietal aroma is described. It is possible that other compounds might have also significant contribution even though not being identified and quantified during the

present research or the odour threshold was not known or available. The identified compounds were divergent from the ones listed in the literature, because of the different quantification methods used, and vintage fluctuations that might cause difference of varietal aroma content.

6.3 Principal components analysis

With the aim to elucidate some relationships between aromatic composition and the studied modalities, a simple and exploratory Principal Components Analysis was performed. PCA was made using the inverse of standard deviation as scaling factor, followed by Cross validation, using Uncertainty test with optimal PC's and a Full Size model.

Figure 26 shows the graph in which the different modalities and the aromatic compounds concentration were projected simultaneously according to PCA. The first three main components PC1, PC2 and PC3 explain 65% of the variance (35%, 16% and 14%, respectively). The first component (PC1) was predominantly characterized by s-α-pinene, ethyl-2-hexenoate and an unidentified terpene, content in the positive side of the axis, and terpinolene, β-damascenone, geranyl acetone and limonene content in the negative side of the same axis. The second component (PC2) was characterized by the three unidentified sesquiterpenes and ethyl dodecanoate content in the positive part of the axis, and α-terpineol, nerolidol and ethyl hexanoate content in the negative part of the axis. The third component (PC3) was characterized by 4-ethylguaiacol, *cis*-β-farnesene, terpinolene, β-damascenone, and limonene content in the positive part of the axis, and *trans*-nerolidol, *trans*-α-bisabol and dihydro pseudo-ionone content in the negative part (Figure 27). Regarding the samples/modalities of the study, PC1 was mainly defined by the modalities of the 2012 vintage in the positive part of the axis, and the modalities of the 2011 vintage in the negative part; PC2 by modalities 2013 vintage in the positive part, and modalities from 2012 and 2011 vintages in the negative part of the axis; finally, PC3 was characterized by modalities from 2011 vintage in the positive part, and DMR from 2012 vintage in the negative part of the axis.

From these multivariate analysis results, we might point a statistical relation between variables, variables and modalities/samples, and between samples/modalities:

Using PC1, an unidentified terpene 1, s-α-pinene and ethyl-2-hexenoate content were related, and also relate with the modalities of the 2012 vintage; terpinolene, β-damascenone, geranyl acetone and limonene content were related and also relate with the modalities of the 2011 vintage;

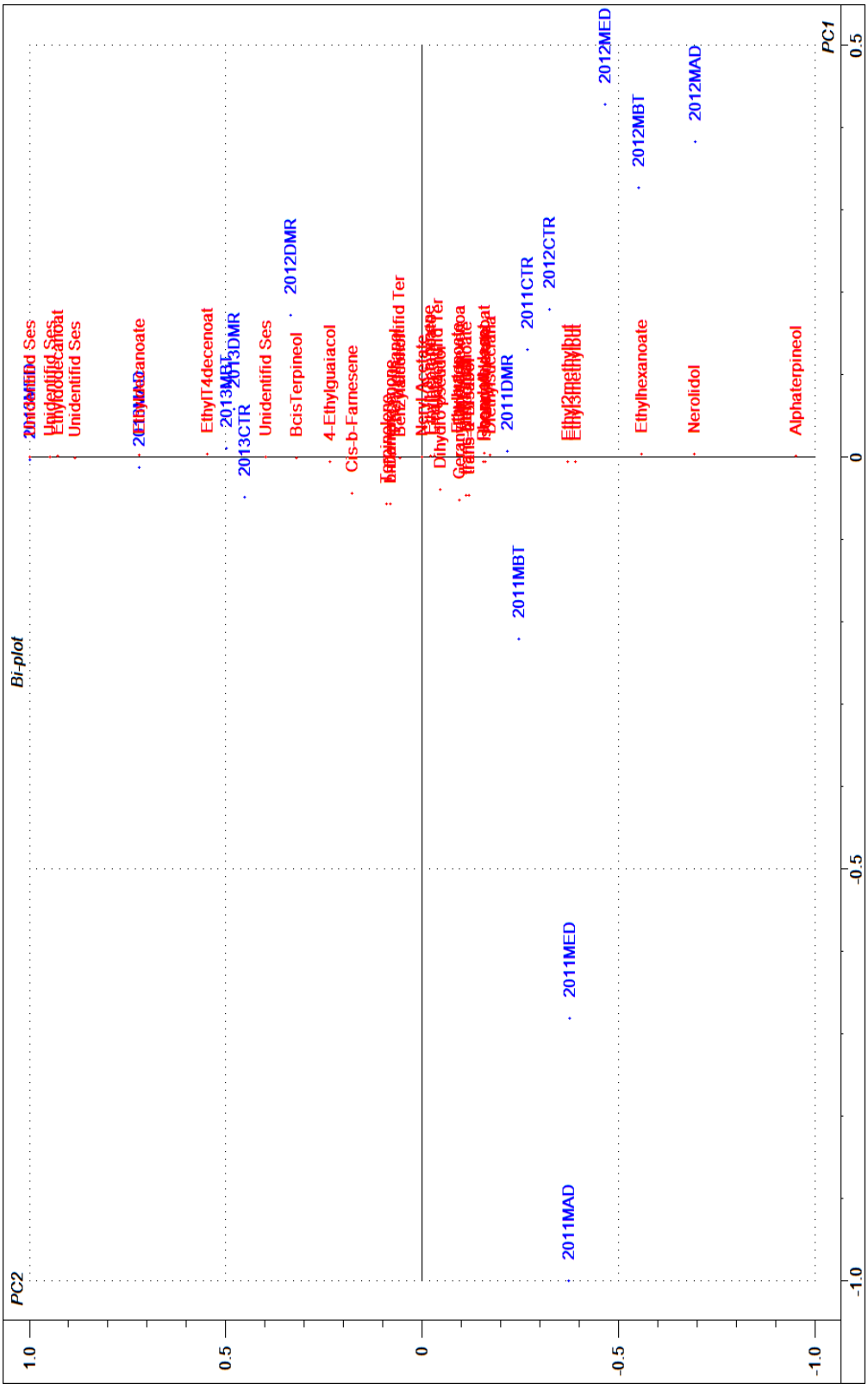

Figure 26 - Principal components analysis (PCA) Scores and Loadings Biplot of aromatic compounds concentration variables and modalities (PC1 35%; PC2 16% of explained variance).

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*').

From PC2, three unknown sesquiterpenes (1,2,3) and ethyl dodecanoate content were related and also relate with modalities 2013 vintage; α-terpineol, nerolidol and ethyl hexanoate content were related and also relate with modalities from 2012 and 2011 vintages;

Using PC3, 4-ethylguaiacol, *cis*-β-farnesene, terpinolene, β-damascenone, and limonene content are related and also relate with modalities from 2011 vintage; *trans*-nerolidol, *trans*-α-bisabolene and dihydro pseudoionone content are related and also relate with DMR from 2012 vintage.

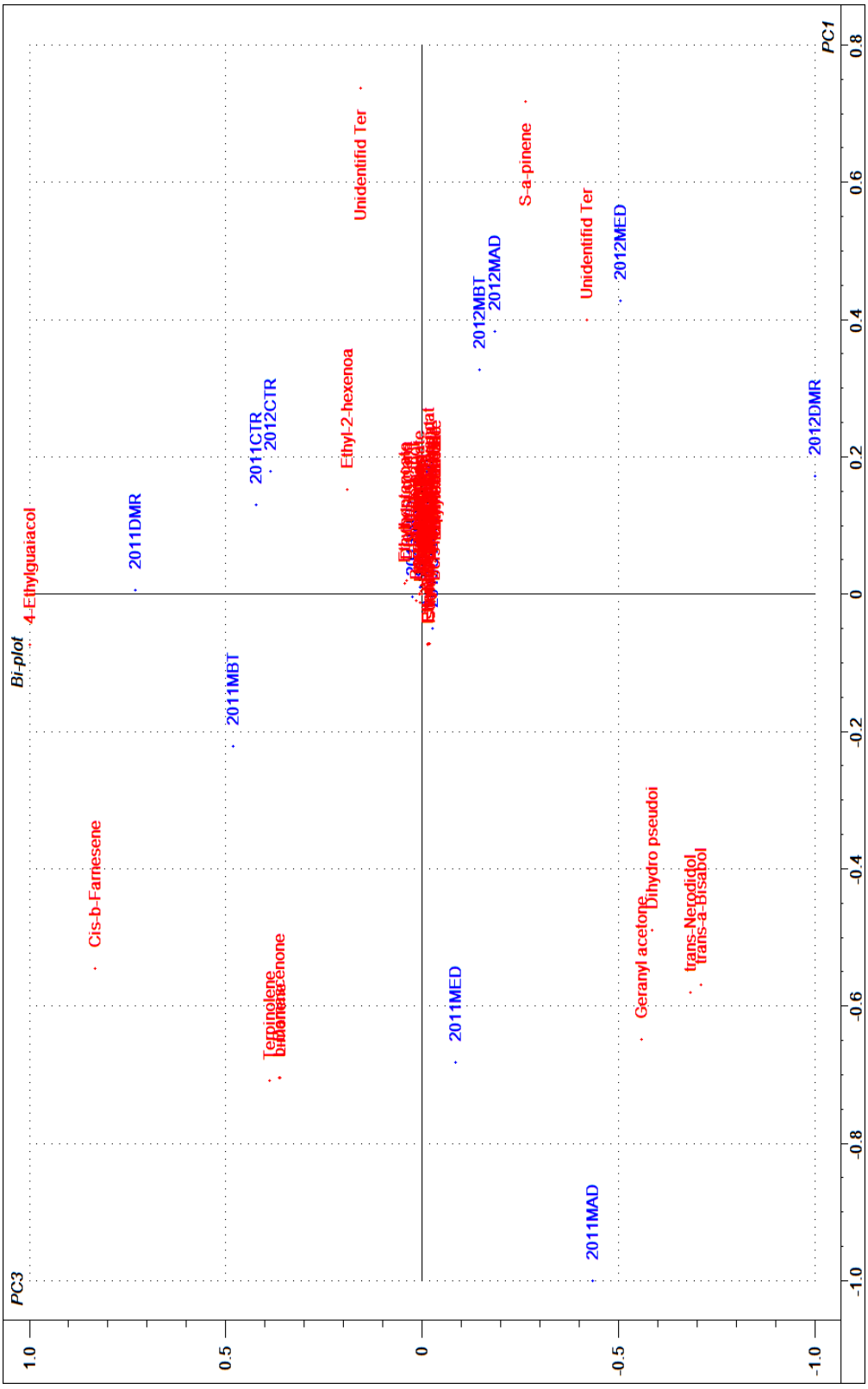

Figure 27 - Principal components analysis (PCA) Scores and Loadings Biplot of aromatic compounds concentration variables and modalities (PC1 35%; PC3 14% of explained variance).

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*').

VII Wine tasting and wine quality

One of the most important aspects of the wine production is the wine quality and the consumer acceptance. This will greatly define the commercial success of the product and the future of a wine company.

The sensorial analysis and tasting of wines produced in the three vintages was performed to simulate both the consumer preferences and also to characterize wines by a more detailed way. The aroma description can be used to characterize the sensorial Baga varietal aroma.

The detailed sensorial wine analysis was performed by 7 tasters expert panel, using a sheet with a score having a scale between 0 to 5 (0 meaning 'non-existent'; 5 meaning 'intense') a list of previously selected aroma descriptors, selected using as varietals for Baga, the aromas reported in literature, magazine and journalist tasting notes. The tasters were asked to give overall score, between 0 and 100, for the global quality of each wine. Sensorial evaluation was limited to aroma analysis.

7.1 Tasting scores

The selected aroma descriptors are: Rose; Violet; Carnation; Apple; Pear; Quince; Tangerine; Grapefruit; Banana; Pineapple; Gooseberry; Raspberry; Cherry; Plum; Blackberry; Dried fruit; Jam; Chestnut; Hazelnut; Almond; Tea; Tobacco; Green Bell pepper; Herbs; Resin; White pepper; Liquorice; Cloves; Vanilla; Toasted; Cedar; Oak; Smoke; Leather; Bread; Yeast; Cream; Butter; Yogurt; Metallic; Honey; Coffee; Chocolate; Mushroom; Rancid/Cheese.

The tasters used a chart (see annex) when tasting with these descriptors and scored each descriptor between 0 and 5 points; if no score was introduced in the tasting chart, it was considered that that descriptor was not present. It was allowed to talk after all the tasters had finished the section. Several commercial Baga varietal wines were tasted between the project wines in order to be used as references (Table 47 and Table 48).

The overall scores were evaluated and only the descriptors considered as 'present' (score above 2) were used for detailed analysis.

Table 47 - Overall Sensorial analysis for wines of 2011, 2012 and 2013 vintages, and references. Average scores.

Descriptor	2011CTR	2011MAD	2011MED	2011MBT	2011DMR	2012CTR	2012MAD	2012MED	2012MBT	2012DMR	2013CTR	2013MAD	2013MED	2013MBT	2013DMR	2013BIO	2013DMR30	11QTA.BAG	13A.SBM	04QTARIG	01CIMPR	91MMCONF	12POEIR	10MESS
Rose	1.4	1.4	2.1	0.7	0.7	1.4	2.1	2.1	2.1	2.8	2.1	2.1	2.8	3.5	3.5	1.4	2.1	0.7	2.1	1.8	1.8	0.9	0.9	1.8
Violet	0.7	0.7	0.7	0.7	2.4	0.7	2.0	2.0	0.7	1.3	0.7	1.4	1.4	0.7	0.0	1.4	1.0	1.4	1.4	1.0	1.0	1.0	2.0	2.0
Clove	0.0	0.0	0.0	1.4	0.7	0.0	0.7	0.7	0.7	0.0	0.0	0.7	0.7	0.7	1.4	0.0	0.0	0.7	0.0	0.9	0.0	0.0	0.0	0.0
Apple	1.4	0.7	0.0	0.0	0.0	1.4	0.0	0.7	0.7	0.0	2.0	0.7	0.0	0.7	1.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pear	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.7	0.0	0.0	0.7	1.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Quince	3.7	3.0	3.3	4.0	1.3	4.0	1.6	1.6	1.2	1.3	3.7	3.0	2.3	4.0	4.0	1.9	1.0	2.0	3.7	1.9	3.0	3.9	3.9	1.1
Tangerine	1.4	0.0	0.0	0.0	0.0	0.0	1.4	0.0	3.0	0.0	0.7	0.7	0.7	1.4	1.4	0.7	1.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Grapefruit	0.7	0.0	0.7	0.7	1.4	2.3	3.4	2.7	1.3	2.4	0.7	0.0	2.1	0.7	0.0	1.4	1.7	0.7	2.4	1.9	1.0	1.0	1.0	1.0
Banana	1.4	0.0	0.0	0.0	0.0	1.0	1.0	1.0	3.1	0.7	1.4	0.7	2.1	0.7	1.4	0.7	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pineapple	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.7	0.0	0.0	0.7	0.0	0.0	0.7	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gooseberry	0.0	0.0	1.4	0.7	0.0	1.4	1.4	0.7	1.4	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	3.6	0.0
Raspberry	1.4	0.7	1.7	0.7	0.7	0.0	2.1	0.7	0.0	1.4	1.4	1.4	1.4	2.1	1.4	2.1	1.7	0.0	1.4	1.9	0.0	0.0	1.9	0.0
Cherry	1.4	0.7	0.0	0.0	0.0	0.7	0.0	0.0	1.4	0.7	1.4	0.0	0.7	0.7	1.4	0.7	1.4	0.0	0.0	0.9	0.0	0.0	4.5	3.6
Plum	0.0	0.0	0.0	0.0	1.4	3.0	0.7	1.4	1.3	1.7	0.7	2.1	1.4	0.7	1.4	2.1	0.0	1.4	2.1	0.0	2.9	1.9	0.0	2.9
Blackberry	0.0	0.0	0.0	1.7	2.3	0.7	0.7	1.4	0.7	0.0	0.7	0.7	0.7	1.4	0.0	2.1	2.3	1.4	1.4	2.1	2.9	2.9	0.0	2.9
Dried plum	1.3	0.0	0.6	2.7	2.7	2.8	1.4	2.8	2.1	1.0	1.4	0.7	1.4	2.8	0.7	2.1	5.0	2.1	1.4	2.1	2.0	3.0	2.0	0.0
Jam	0.0	0.0	0.0	0.0	1.6	0.0	0.7	0.7	0.0	1.4	0.0	0.0	1.4	0.7	1.4	0.7	2.0	0.0	0.0	0.0	1.1	1.9	1.1	0.0
Nut	0.0	0.7	0.0	0.0	0.0	1.4	0.0	0.7	0.7	0.0	1.4	0.7	0.7	2.1	0.7	0.0	0.7	0.0	0.0	0.9	1.8	0.0	0.0	0.9
Hazelnut	1.4	0.0	1.4	0.0	1.4	0.7	2.3	4.0	2.6	0.3	0.7	1.4	2.1	0.7	0.7	1.4	3.0	0.7	1.4	1.1	1.1	0.0	0.0	0.0
Almond	0.0	0.0	0.0	1.4	0.0	0.0	0.0	0.7	1.4	0.0	0.0	0.0	0.0	1.4	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0
Tea	0.0	0.0	0.7	0.0	2.1	1.4	0.7	1.4	0.7	0.7	2.1	1.4	1.4	2.8	2.1	1.4	2.8	0.7	0.0	1.8	1.8	1.8	1.8	0.0
Tobacco	0.7	0.0	1.4	0.0	3.4	2.1	3.4	2.3	1.6	1.3	2.0	2.0	1.0	3.7	3.3	2.3	4.0	0.7	1.4	2.0	2.0	1.0	1.0	0.0
Bell pepper	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Herbs	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.7	1.8	3.6	2.7
Resin	1.7	2.1	2.8	1.4	0.0	1.4	1.3	1.6	2.3	1.7	2.1	2.1	1.4	2.8	2.8	1.4	2.0	1.4	2.0	2.9	3.9	3.9	4.9	2.9
White pepper	0.7	0.0	2.1	0.7	0.0	0.7	0.7	0.7	0.7	0.7	0.7	0.0	1.4	0.7	0.7	0.7	0.7	0.0	2.1	0.9	3.6	3.6	0.9	1.8
Licorice	0.0	0.0	0.0	0.0	3.4	1.4	2.8	2.1	0.0	0.7	0.0	0.0	2.1	0.7	0.7	0.0	0.0	1.4	1.4	1.9	0.0	2.1	2.1	2.0
Red clove	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.9	0.0
Vanilla	0.0	0.0	0.7	0.7	0.7	0.7	2.0	0.7	1.3	0.3	0.0	0.7	1.4	0.0	0.7	0.7	0.0	1.7	1.4	4.0	3.0	0.0	0.0	3.8
Toasted	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.8	1.8	1.8	0.0	0.0
Cedar	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Oak	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.8	0.9	0.9	0.0	0.0
Smoke	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.7	0.0	0.0	0.7	0.0	0.0	0.0	2.0	0.0	0.0	1.1	0.0	0.0	1.0	1.1
Leather	1.4	0.7	2.1	1.2	0.7	1.4	0.7	0.7	1.4	3.7	1.4	2.1	2.1	1.4	0.7	1.4	1.0	4.4	3.8	1.0	1.1	3.0	1.1	0.0
Bread	0.7	0.7	0.7	0.7	0.7	0.7	0.7	1.4	2.1	1.3	1.4	1.4	2.1	1.4	2.1	1.4	2.0	0.7	0.7	0.0	0.0	0.0	0.0	0.0
Yeast	0.7	0.7	0.7	0.7	0.7	1.4	0.7	1.3	1.3	1.3	0.7	0.7	0.7	0.7	0.7	0.7	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cream	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Butter	0.0	0.0	0.0	0.0	2.0	0.7	1.4	2.4	2.0	1.3	0.0	0.7	0.0	0.0	0.0	0.0	0.0	1.7	1.0	0.0	1.1	0.0	0.0	0.0
Yogurt	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Metalic	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Honey	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.8	0.0	0.0	0.9	0.0
Coffee	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.8	2.7	0.0	0.9	0.0
Chocolat	1.4	0.0	0.0	0.0	0.0	0.7	0.0	0.0	2.7	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.9	3.0
Mushroom	2.3	2.7	3.0	4.7	2.3	2.7	2.7	2.7	3.7	2.7	3.0	2.7	2.0	3.7	3.7	3.3	2.0	4.4	1.4	1.1	3.9	3.8	0.0	1.1
Cheese	1.0	3.0	2.1	0.7	0.0	2.3	0.0	0.0	0.0	0.7	2.0	1.4	0.7	0.7	1.4	1.4	1.0	0.7	0.7	0.0	0.0	0.0	0.0	0.0
Score	60.0	71.5	71.5	68.0	86.5	73.5	78.5	78.5	69.4	81.5	61.5	66.5	78.5	75.0	75.1	78.5	76.5	77.9	68.5	80.0	75.0	85.0	70.0	90.0

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*').

Table 48 - Scores from selected descriptors. Average sensorial analysis scores for wines by modality and vintages, and all Baga commercial wine references.

Descriptor	2011CTR	2011MAD	2011MED	2011MBT	2011DMR
Rose	1,4	1,4	2,1	0,7	0,7
Violet	0,7	0,7	0,7	0,7	2,4
Quince	3,7	3,0	3,3	4,0	1,3
Grapefruit	0,7	0,0	0,7	0,7	1,4
Plum	0,0	0,0	0,0	0,0	1,4
Blackberry	0,0	0,0	0,0	1,7	2,3
Dried fruits	1,3	0,0	0,6	2,7	2,7
Tea	0,0	0,0	0,7	0,0	2,1
Tobacco	0,7	0,0	1,4	0,0	3,4
Resin	1,7	2,1	2,8	1,4	0,0
Pepper	0,7	0,0	2,1	0,7	0,0
Leather	1,4	0,7	2,1	1,2	0,7
Mushroom	2,3	2,7	3,0	4,7	2,3
Cheese	1,0	3,0	2,1	0,7	0,0
SCORE	60,0	71,5	71,5	68,0	86,5

Descriptor	2012CTR	2012MAD	2012MED	2012MBT	2012DMR
Rose	1,4	2,1	2,1	2,1	2,8
Violet	0,7	2,0	2,0	0,7	1,3
Quince	4,0	1,6	1,6	1,2	1,3
Grapefruit	2,3	3,4	2,7	1,3	2,4
Plum	3,0	0,7	1,4	1,3	1,7
Blackberry	0,7	0,7	1,4	0,7	0,0
Dried fruits	2,8	1,4	2,8	2,1	1,0
Tea	1,4	0,7	1,4	0,7	0,7
Tobacco	2,1	3,4	2,3	1,6	1,3
Resin	1,4	1,3	1,6	2,3	1,7
Pepper	0,7	0,7	0,7	0,7	0,7
Leather	1,4	0,7	0,7	1,4	3,7
Mushroom	2,7	2,7	2,7	3,7	2,7
Cheese	2,3	0,0	0,0	0,0	0,7
SCORE	73,5	78,5	78,5	69,4	81,5

Descriptor	2013CTR	2013MAD	2013MED	2013MBT	2013DMR	REFS
Rose	2,1	2,1	2,8	3,5	3,5	1,4
Violet	0,7	1,4	1,4	0,7	0,0	1,4
Quince	3,7	3,0	2,3	4,0	4,0	2,8
Grapefruit	0,7	0,0	2,1	0,7	0,0	1,3
Plum	0,7	2,1	1,4	0,7	1,4	1,6
Blackberry	0,7	0,7	0,7	1,4	0,0	1,9
Dried fruits	1,4	0,7	1,4	2,8	0,7	1,8
Tea	2,1	1,4	1,4	2,8	2,1	1,1
Tobacco	2,0	2,0	1,0	3,7	3,3	1,2
Resin	2,1	2,1	1,4	2,8	2,8	3,1
Pepper	0,7	0,0	1,4	0,7	0,7	1,8
Leather	1,4	2,1	2,1	1,4	0,7	2,1
Mushroom	3,0	2,7	2,0	3,7	3,7	2,2
Cheese	2,0	1,4	0,7	0,7	1,4	0,2
SCORE	61,5	66,5	78,5	75,0	75,1	78,1

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*').

Figure 28 and Figure 29 display the sensorial scores averages for the modalities and vintages, with comparison with the reference wines. Wines from CTR modalities were described as having perceptive notes (high scores) for 'Quince', 'Mushroom', 'Dried fruits', 'Resin', and 'Rose', and scoring 65 points out of 100 for overall quality. MAD wines were defined as having perceptive notes for 'Mushroom', 'Quince', 'Rose', 'Tobacco', 'Resin', and scoring 72 points out of 100 for overall quality. Wines from MED modalities were characterized as having perceptive notes of 'Mushroom', 'Quince', 'Rose', 'Resin', 'Grapefruit', Dried fruits', 'Tobacco', 'Leather', and scored 76 points out of 100 for overall quality. As for MBT wines, it were described as having perceptive

notes of ‘Mushroom’, ‘Quince’, Dried fruits’, ‘Resin’, Rose’, ‘Tobacco’, and having scored 71 points out of 100 for overall quality. DMR wines were described as having perceptive notes of ‘Mushroom’, Tobacco’, ‘Rose’, ‘Quince’, Leather’, ‘Tea’, ‘Plum’, ‘Dried fruits’ and ‘Resin’, and having scored 81 points out of 100 for overall quality. As for the Baga wines References, they had perceptive notes of ‘Resin’, ‘Quince’, ‘Mushroom’, ‘Leather’, ‘Blackberry’, ‘Dried fruits’, ‘Pepper’ and ‘Plum’, having scored 78 points out of 100 for overall quality.

Although the most important descriptors were almost the same for all the modalities, their intensity and overall richness were quite diverse and their influence over the wine quality was different. ‘Mushroom’, ‘Quince’ ‘Dried fruits’, ‘Resin’ and ‘Leather’, for example, are descriptors described by the tasters as positive notes if low perceptive quantities but unpleasant if having stronger perception. On contrary, ‘Violet’, ‘Grapefruit’, ‘Plum’, ‘Blackberry’ were described as being interesting if present in the wine perceptive notes, even if present in high intensities. Other important aspect is the complexity and richness of the aroma. A more complex aroma, with more descriptors identified and with perceptive notes, might be more pleasant and interesting, resulting in overall higher scores. The more unpleasant notes might be attenuated in a complex wine aroma matrix because their unpleasant influence could be masked in the complex matrix.

On average, the wines from DMR and MED modalities were scored as the most attractive, with DMR2011 and DMR2012 wines having higher scores for overall quality than the used commercial wine References. MAD2012, MED2012 and MED2013 showed overall scores above the References, and these results displayed the potential of DMR, MED and MAD to produce high quality Baga wines, and even the ability to enhance the perceptive quality above the actual production methods.

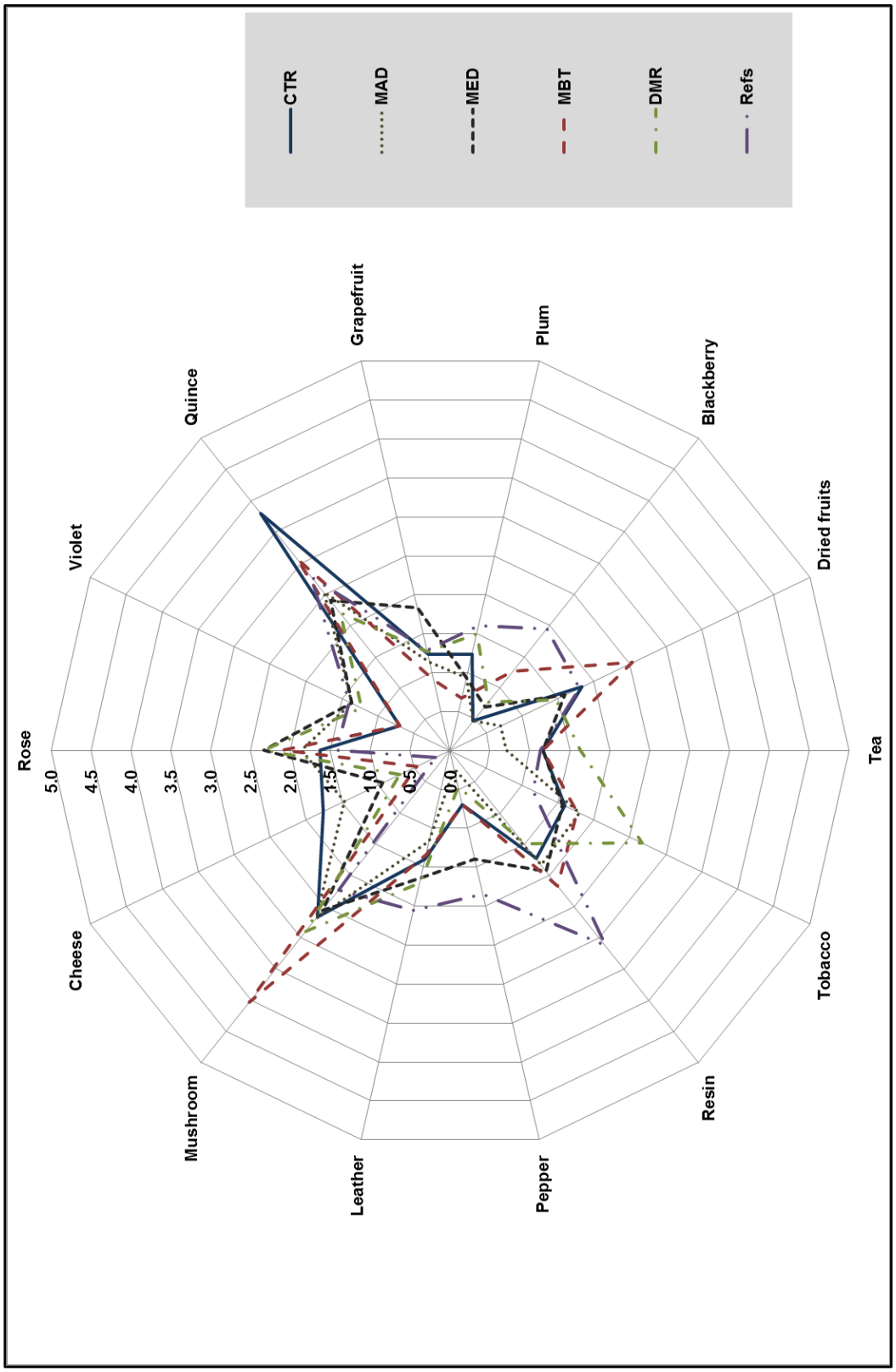

Figure 28 - Average sensorial analysis scores from selected descriptors, for wines by modality and references.

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*').

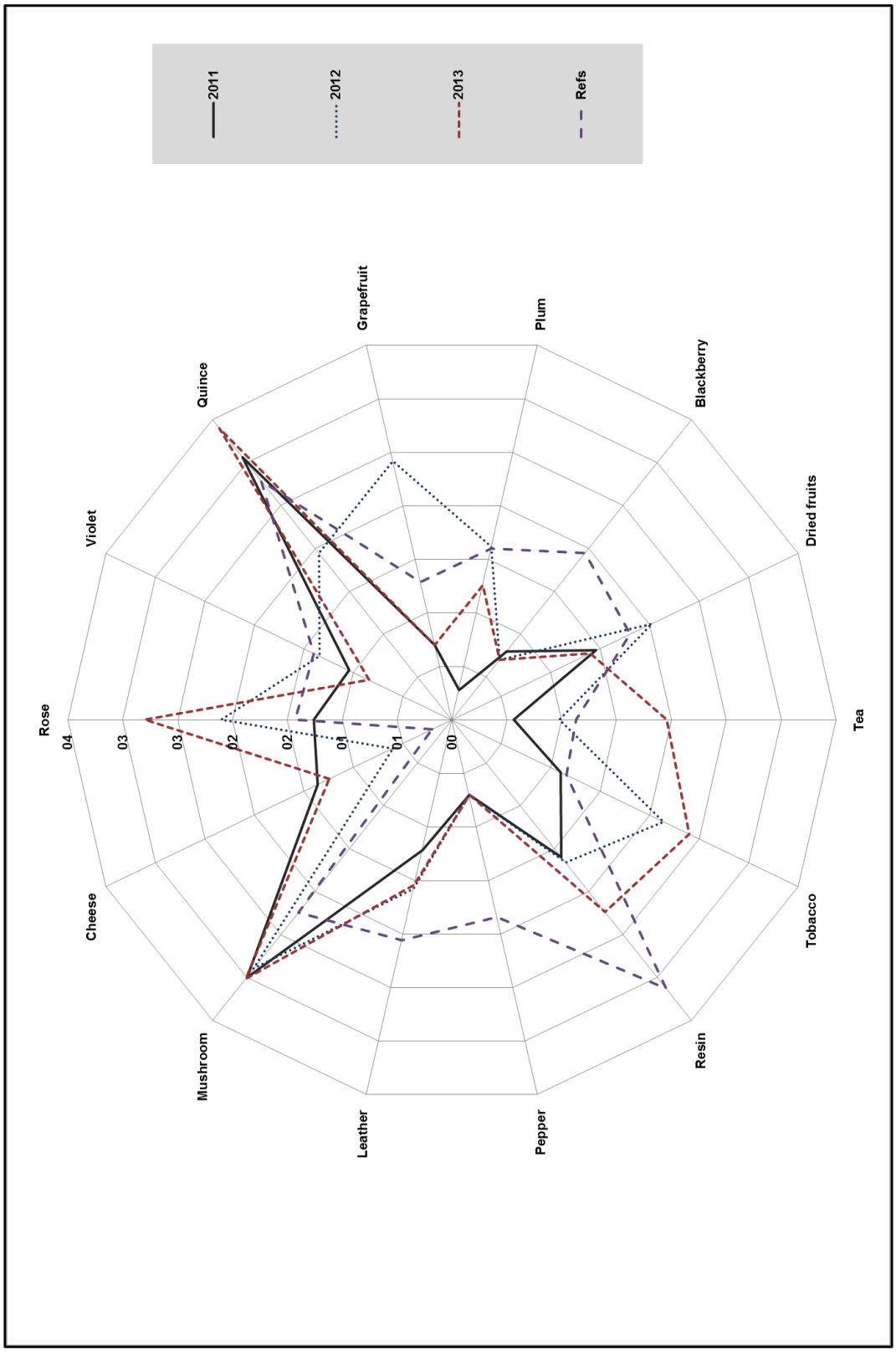

Figure 29 - Average sensorial analysis scores, from selected descriptors, for wines by vintage and references.

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*').

7.2 Principal components analysis (PCA)

Aiming to clarify some relationships between sensorial data and the studied modalities, a simple and exploratory Principal Components Analysis was performed. PCA was made using the inverse of standard deviation as scaling factor, followed by Cross validation, using Uncertainty test with optimal PC's and a Full Size model.

Figure 30 illustrates the graph in which the different modalities and the Selected Sensorial evaluation variables (descriptors and wine scores) were projected simultaneously according to PCA. The first three main components PC1, PC2 and PC3 explain 66% of the variance (33%, 17% and 16%, respectively). The first component (PC1) was predominantly characterized by the variables 'Resin', 'Cheese' and 'Quince' descriptors in the positive side of the axis, and 'Violet', 'Blackberry', 'Grapefruit' descriptor and the Overall Score, in the negative side of the axis. The second component (PC2) was characterized by the 'Rose', 'Tea', 'Tobacco', 'Resin' and 'Plum' descriptors in the positive part of the axis, and 'Violet', 'Cheese' and 'Grapefruit' descriptors in the negative part of the axis. The third component (PC3) was characterized by 'Leather', 'Rose', 'Grapefruit' and 'Pepper' descriptors and the Score in the positive part of the axis, and 'Mushroom', 'Blackberry', 'Dried fruit', and 'Quince' descriptors in the negative part (Figure 31). Regarding the samples/modalities of the study, PC1 was mainly defined by the DMR, MAD and MED modalities in the positive part of the axis, and the CTR and MBT modalities in the negative part; PC2 by modalities from 2013 vintage in the positive part, and modalities from 2011 vintage in the negative part of the axis; finally, PC3 was characterized by DMR, MAD and MED modalities in the positive part, and MBT and CTR modalities in the negative part of the axis.

From these multivariate analysis results, we might point a statistical relation between variables, variables and modalities/samples, and between samples/modalities:

Using PC1, 'Resin', 'Cheese' and 'Quince' descriptors were related, and also relate with the DMR, MAD and MED modalities; 'Violet', 'Blackberry', 'Grapefruit' descriptors and the Overall Score were related and also relate with the modalities from 2011 vintage;

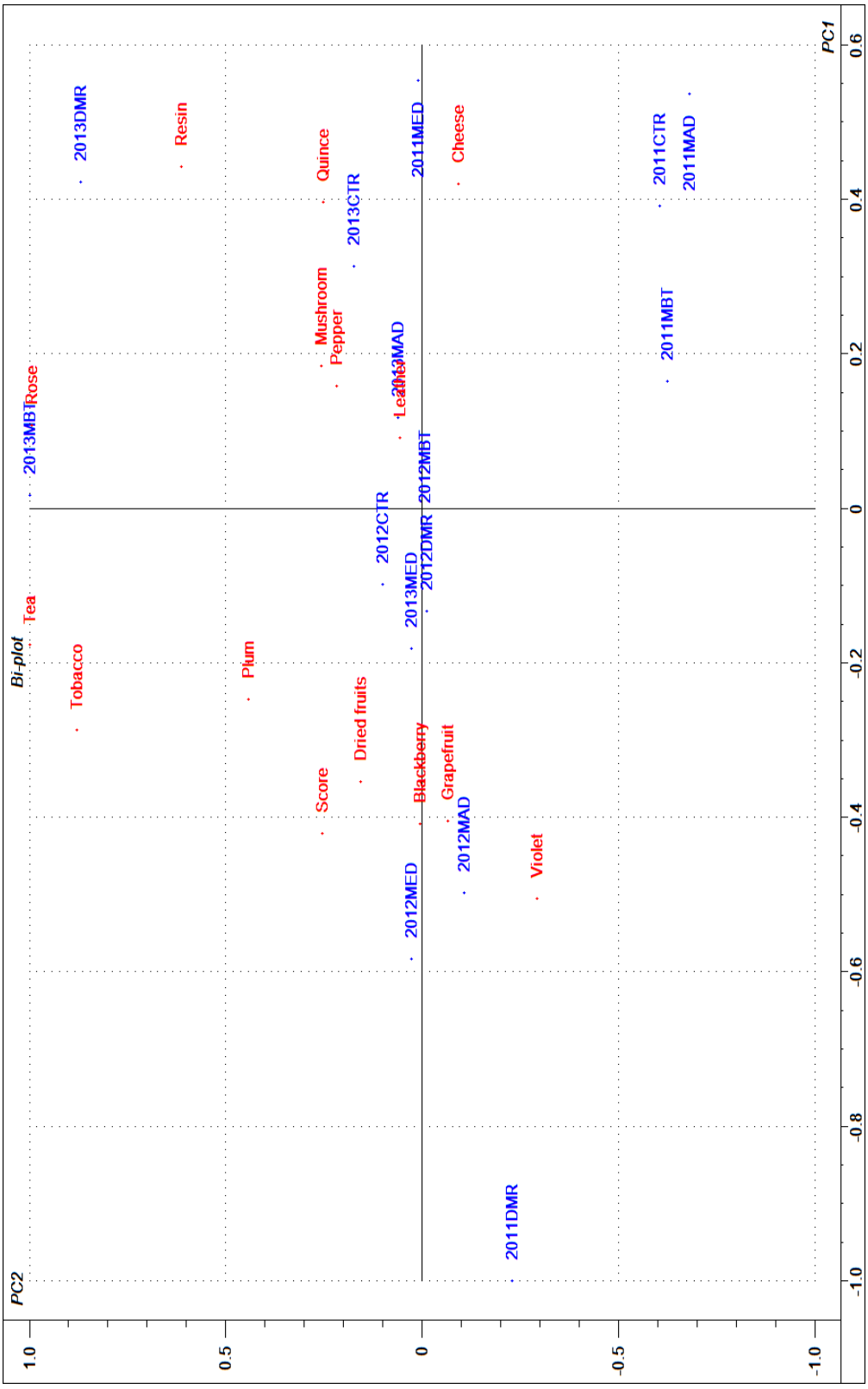

Figure 30 - Principal components analysis (PCA) Scores and Loadings Biplot of sensorial evaluation variables and modalities (PC1 33%; PC2 17% of explained variance).

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*').

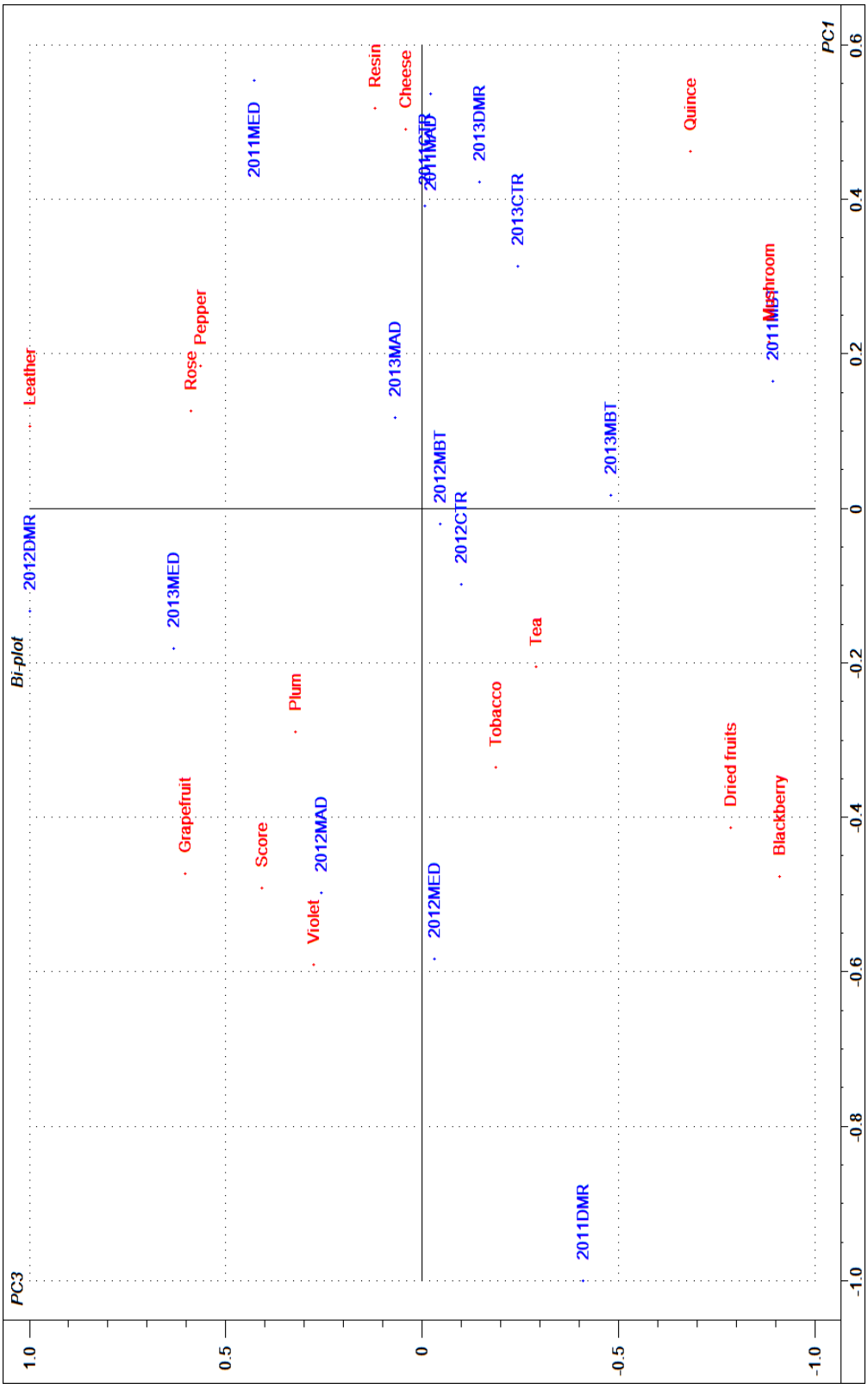

Figure 31 - Principal components analysis (PCA) Scores and Loadings Biplot of sensorial evaluation variables and modalities (PC1 33%; PC2 16% of explained variance).

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*').

From PC2, ‘Rose’, ‘Tea’, ‘Tobacco’, ‘Resin’ and ‘Plum’ descriptors were related and also relate with modalities from 2013 vintage; ‘Violet’, ‘Cheese’ and ‘Grapefruit’ descriptors were related and also relate with modalities from 2011 vintage;

Using PC3, by ‘Leather’, ‘Rose’, ‘Grapefruit’ and ‘Pepper’ descriptors and the Overall Score were related and also relate with DMR, MAD and MED modalities; ‘Mushroom’, ‘Blackberry’, ‘Dried fruit’, and ‘Quince’ descriptors are related and also relate with MBT and CTR modalities.

VIII Broad perspective over results and wine quality

The experimental work was performed in a wine company context, with no environmental control, having the drawback that results are subjected to variation from one vintage to the other, due essentially to climatic conditions. This fact can be a significant limitation when performing research thus obtaining robust results can take several years of experiments. However considering this limitation the work can still help to understand the context of making decisions in the vineyard, where the reality is like this.

It was decided to perform a Principal Component Analysis using the variables that appeared to contribute most for results variance of previous PCA (%GAPS, %IC, LLN, Cluster weight, Number of Berries, Cluster compactness, *Botrytis* incidence, Yield, LA/Yield ratio, Ravaz index, Skin/pulp ratio, Brix degree, juice pH, Titratable acidity, Sugar load, Anthocyanin concentration, Seed number, Carotenoids and Chlorophylls compounds, aromatic compounds with significant OAV). With the aim to get some more information about relations between the most important variables for results variance and the studied modalities, a simple and exploratory Principal Components Analysis was performed, using the inverse of standard deviation as scaling factor, followed by Cross validation, using Uncertainty test with optimal PC's and a Full Size model.

Figure 32 shows the graph in which the different modalities and the high impact variables were projected simultaneously according to PCA. The first four main components PC1, PC2, PC3 and PC4 explain 77% of the variance (43%, 17%, 10%, and 7% respectively). The first component (PC1), that explained 43% of total variance, was predominantly characterized by having strong influence of %GAPS, LA/Yield ratio, anthocyanin concentration, juice pH, Skin/pulp ratio and Brix degree in the positive part of the axis; Ravaz index, Seed number, LLN, chlorophyll b content, lutein content, Yield, Cluster weight influence in the negative side of the axis. The positive part is influenced by 2013 samples and the negative part by 2012 samples.

The second component (PC2), that explained 17% of total variance, was characterized by Cluster compactness, β-damascenone content, linalool content, Number of berries, Fruit set, Cluster weight in the positive side of the axis; Titratable acidity, Brix degree, Skin/pulp ratio content, %IC, lutein content, chlorophyll b content show more influence in the negative part of the axis. The positive part is also influenced by 2013 samples and the negative part by DMR samples.

The third component (PC3), explained 10% of total variance, and was predominantly characterized by having strong influence of *Botrytis* incidence, Anthocyanin concentration, Sugar load and pheophytin content in the positive part of the axis; Number of berries, Fruit set, Skin/pulp ratio and Cluster weight influence in the negative side of the axis. The positive part is influenced by 2011 and 2013 MED samples and the negative part by 2013 and 2012 samples.

The fourth component (PC4), explained 7% of total variance, and was predominantly characterized by having strong influence of *Botrytis* incidence, %IC, Cluster compactness, LLN and pH in the positive part of the axis; pheophytin content, nerolidol concentration and Number of seeds influence in the negative side of the axis. The positive part is influenced by CTR and MBT samples and the negative part by MAD and MED samples.

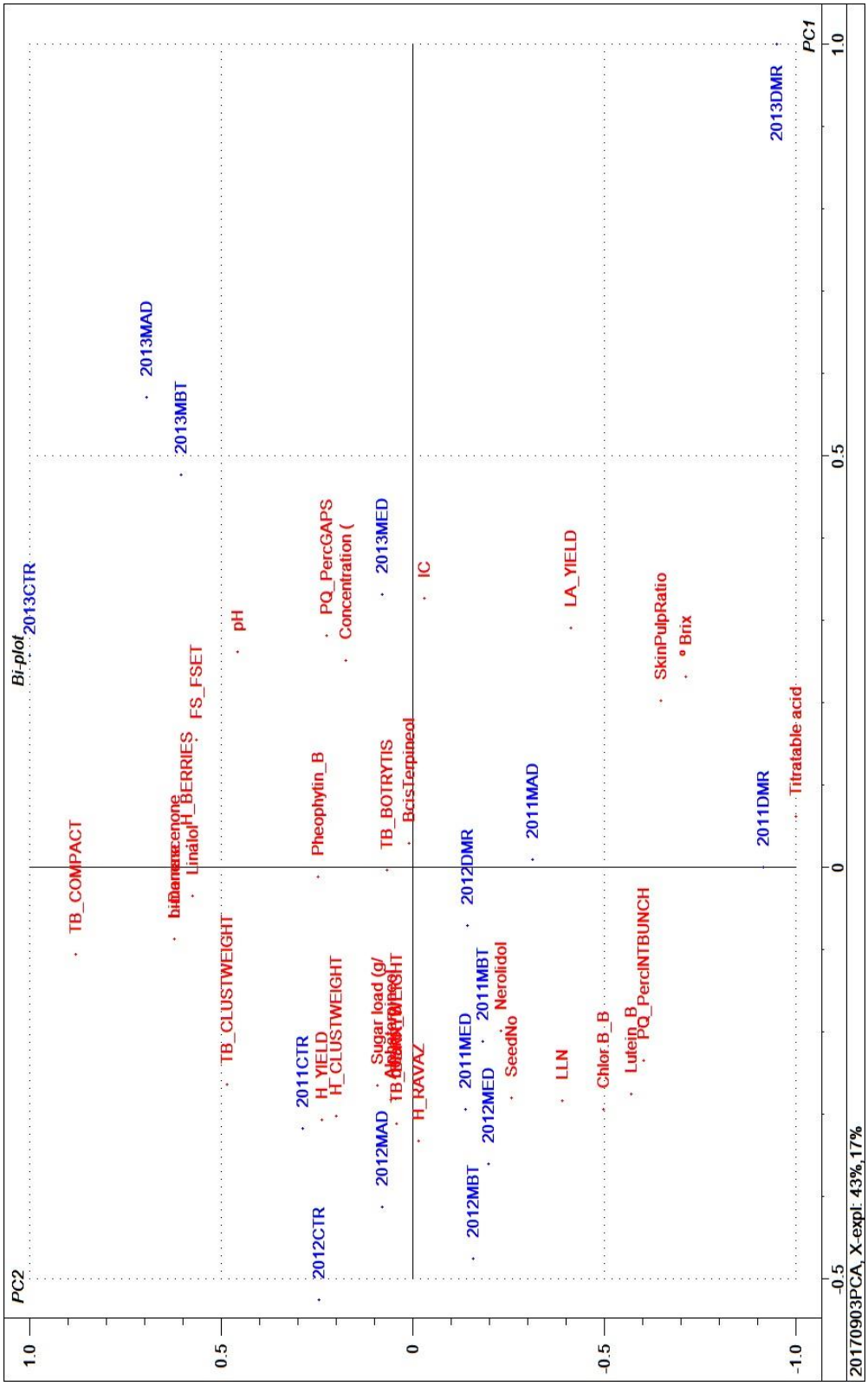

Figure 32 - Principal components analysis (PCA) Scores and Loadings Biplot of impact variables and modalities (PC1 43%; PC2 17% of explained variance).

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*').

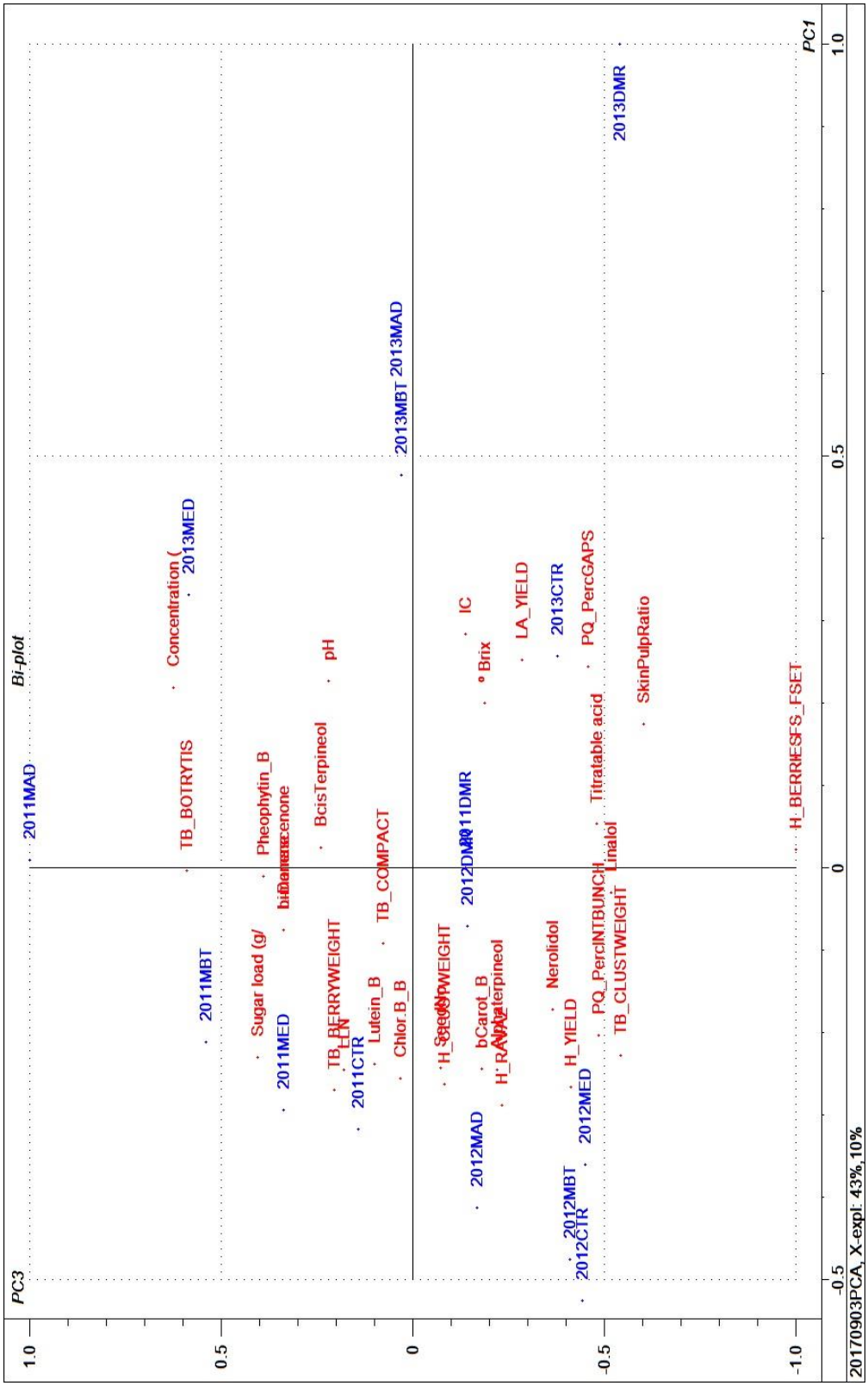

Figure 33 - Principal components analysis (PCA) Scores and Loadings Biplot of impact variables and modalities (PC1 43%; PC3 10% of explained variance).

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*').

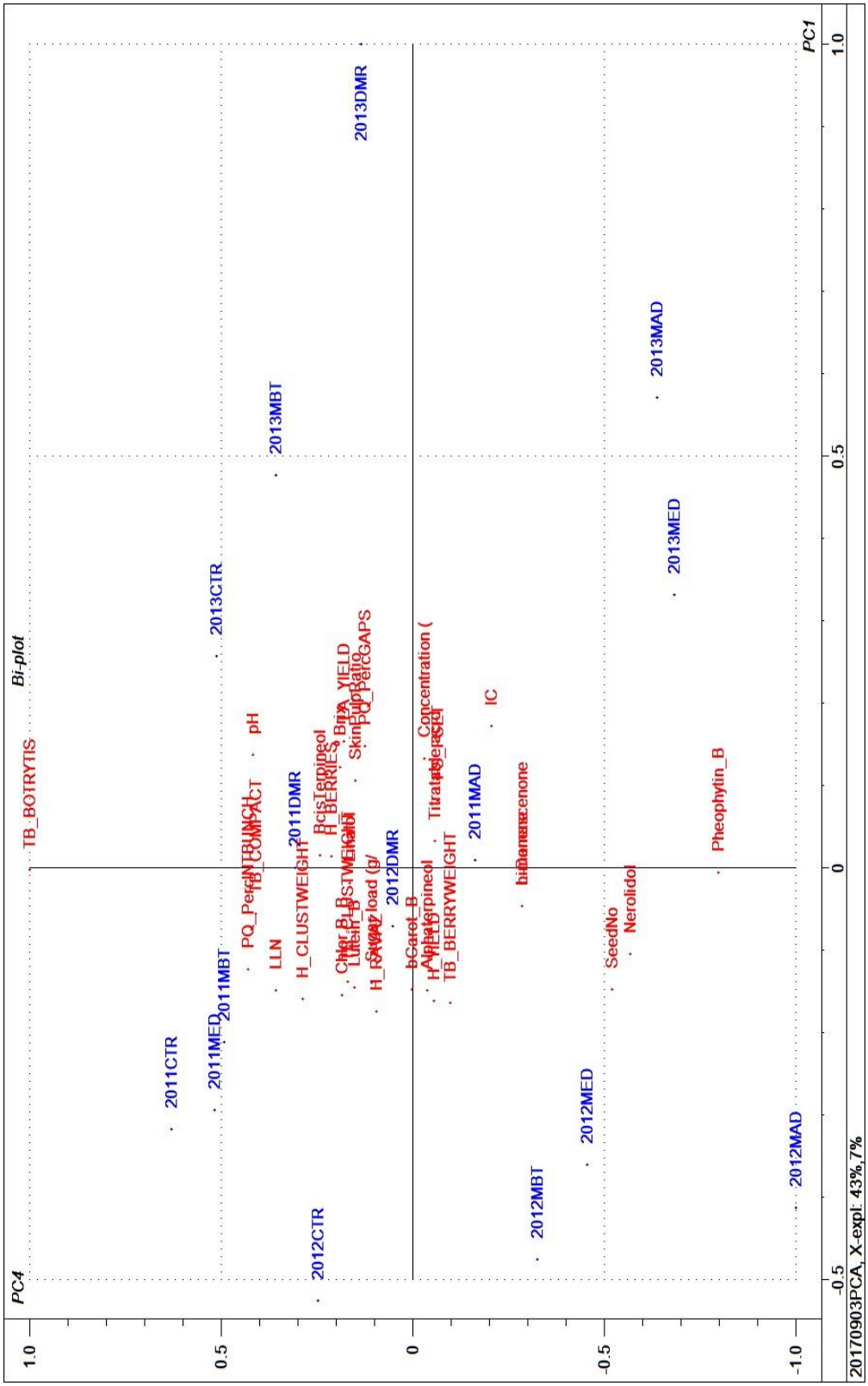

Figure 34 - Principal components analysis (PCA) Scores and Loadings Biplot of impact variables and modalities (PC1 43%; PC4 7% of explained variance).

CTR (control); MAD (Manual early defoliation); MED (Mechanical early defoliation); MBT (Manual bunch thinning); DMR ('*Double maturation raisonnée*').

Using a broad perspective over the results, with the concern of establishing a correlation between the viticultural conditions and the grape characteristics as the most important assets for wine production and wine quality, the results were interesting and not entirely expected.

Summarizing:

All the studied modalities reduced yield, as expected.

MAD and MED results proved that temporary limitation of photosynthates during flowering causes berry abortion and fruit set reduction, only forming the fit berries, confirmed by the higher number of seeds produced. Both techniques reduced yield, with MAD being time consuming and labour demanding, and MED needing the use of machinery in early moment of the vegetative growth and may cause flowers and clusters damage.

MBT reduced yield by decreasing the number of clusters in the vine. Besides being time consuming and labour demanding, MBT results showed to be unpredictable and inconsistent - the impact over yield reduction varies because of berry weight/volume compensation, which may also cause the dilution of berry compounds, resulting in a decrease of the quality of grapes. Moreover, at the time when cluster thinning is executed, there was an instantaneous increase of grape quality - if the less interesting grapes with lower quality or showing incidence of disease were removed, consequently, the average of the remaining fruits will have an improved quality, straightaway.

DMR reduced yield by sunlight induced dehydration of the fruits. Besides the vast labour demand, and time consumption, there was a significant risk of losing yield due to separation of berries from the clusters caused by dehydration, combined with the fact of having a short ripening process, interrupted by performing DMR in the vine.

Apart from the viticultural aspects of the research, there was also the consumer point of view, the quality perception of the wines. Even though alcoholic fermentations were not implemented in optimal conditions (small batches of juice fermenting, poor temperature control, malolactic fermentation difficult to undergo in some cases), and the fact that the wines were tasted at the same time in 2014 (wines from 2011, 2012 and 2013 vintages), some data could even be taken from sensory analysis and it was elucidative for the quality determination of wines and to draw some conclusions of the possible impact of each modality in wine sensorial performance. DMR wines were

those that have obtained the highest global score, highly appreciated by all expert tasting panel. Comparing with the Baga References wines average score, which have obtained 78.1/100 points, DMR wines scored 86.5, 81.5 and 75.1 points out of 100 (average from 3-vintage, 81.0 points). Wines from MED were also obtained high score (3-vintage average of 76.2 points), a bit lower than References wines average score, with 2012 and 2013 wines scoring 78.5 points. MAD and MBT scored lower (72.2 and 70.8 respectively), and the lowest average was for CTR (65.0 points).

Highest scores were closely related with 'Quince' and 'Mushroom' notes - intense perception of these two descriptors result in poorly scored wines. 'Quince' is typically related with unripe fruits, and 'Mushroom' with bacterial spoilage. 'Resin' is associated with unripe fruits as well, but it is also a typical Baga descriptor. Nevertheless, intense perception of this descriptor might be negative. 'Rose' and 'Grapefruit' are positive descriptors and associated with ripe fruits and positive scores, and also 'Plum' and 'Violet' in smaller extent. The wines with better appreciation from the panel combine lower scores for unripe fruit descriptors and higher scores for ripe fruit descriptors.

Relating both viticultural and fruit/wine quality perception, early leaf removal and '*Double maturation raisonnée*' might represent quality improvement for Baga variety, even regarding vines where cluster thinning is usually performed. There is no clear quality improvement of grapes and wines when performing cluster thinning was done. The labour cost might be too high (40 to 70 hours per hectare) to MBT effectively be positive for grape and wine production. There is a clear risk that the investment in labour will produce no superior results. Even though the labour cost of early leaf removal and DMR are also be high (around 40 hours per hectare to MAD, and 50 to 70 hours per hectare for DMR), the positive results might overcome the costs.

IX Conclusions and Final Remarks

In order to systematize the conclusions, they will be grouped taking into consideration each of the studied modalities.

The conclusions about the present research thesis referring to manual early severe defoliation (MAD) are the following:

Yield components

MAD reduced significantly the grape production, by diminishing the fruit set (not significantly) and, therefore, the number of berries (significantly) - it produced lighter and looser clusters (both significantly). Cluster weight was significantly lower than CTR, Berry weight was not significantly affected by MAD (either using marked cluster or average weight at harvest), nevertheless lower weight than CTR was observed, and the cluster was significantly less compact. The effects of MAD on yield have been annual, overlapping climate effects occurring each year. These effects should not be considered cumulative since there was not a significant variation of bud fertility the subsequent years. The reduction of fruit set (not significant), cluster weight and yield (significant) show that the restriction of sources of carbohydrates during the flowering and fruit set might be an effective strategy to control yield. MAD altered the distribution of the components of the berry (skin with less weight and pulp with higher weight) but did not increase the skin/pulp ratio when compared with CTR. The number of seeds for MAD was higher than CTR, which can mean that only the most viable flowers are converted into berries. The leaf area to yield ratio was significantly similar to CTR.

Vegetative growth and health status

MAD removed 46 to 56% of the leaf area of the vines and laterals growth compensates removed leaf area until *veraison*. The dimensions of the canopy did not alter significantly over the three vintages when compared with CTR. Bud break and Potential Fertility indexes did not differ significantly from CTR. MAD did not alter the porosity of the canopy, the number of leaf layers or the interior leaves percentage - the removed leaf area was compensated until *veraison*. The percentage of interior cluster was significantly lower, increasing the light exposure of the clusters. Ravaz index was significantly similar to CTR but the values were always lower. MAD vines displayed significantly lower incidence of *Botrytis*. The combination of more exposed clusters and less compact clusters induced to lower disease incidence.

Grape composition and wine quality

MAD grapevines produced grapes with higher Brix degree than CTR (Probable alcohol) and similar acidity, with less weight and less volume berries. The estimate

Sugar Load for MAD grapes was higher than CTR, which means that the ripening was in a more advanced stage. Adding to this, grape juice displayed higher colour intensity, anthocyanins concentration per berry and total polyphenols are also higher. Better light exposure and cluster microclimate, combined with looser clusters and lower disease incidence might be the reason of the improved grape quality for MAD. MAD grapes showed to have higher content of carotenoids per berry, probably resulting from the better cluster microclimate and light exposure. MAD wines displayed less concentrations of esters, alcohols and sesquiterpenes, and higher concentrations of terpenes, norisoprenoids. This may result in more intense aromatic notes.

Wine sensorial perception of quality

MAD wines have had higher overall score than CTR wines. MAD wines showed to have descriptors like ‘Mushroom’, ‘Quince’, ‘Rose’, ‘Tobacco’ and ‘Resin’ mentioned as perceptive and intense. MAD wines benefit from having a lower *Botrytis* incidence and so they displayed higher sensorial scores and consumer acceptance.

These obtained results indicate that lower yield might be obtained from MAD and that this technique might be capable of improving the oenological and potential consumer acceptance of Baga wines. It must be remembered that MAD is a labour demanding technique and time consuming as well.

The conclusions about the present research work referring to mechanical early severe defoliation (MED) are:

Yield components

MED reduced significantly the grape production, by reducing the fruit set (not significantly) and, therefore, the number of berries (significantly) - it produced lighter and looser clusters (both significantly). Cluster weight was significantly lower than CTR (either using marked cluster or average weight at harvest), Berry weight was significantly inferior than CTR, and the cluster was significantly less compact. The effects of MED on yield have been annual, overlapping the climate effects of the year. There should not be cumulative effects since there was not a significant variation of bud fertility the following years. The reduction of fruit set (not significantly) and the other consequences of yield components might display that the restriction of sources of carbohydrates during the flowering and fruit set might be an effective strategy to control yield. Additionally, there could be also the effect of flower and cluster destruction by leaf removal machinery, which might cause lowering of fruit set and yield. MED altered the distribution of the components of the Berry (skin with less weight and pulp with higher weight) but did not increase the skin/pulp ratio when compared with CTR. The number of seeds for MED was higher than CTR, which might mean that only the more

viable flowers are converted into berries. The leaf area to yield ratio was significantly similar to CTR.

Vegetative growth and health status

MED removed 22 to 30% of the leaf area of the vines and laterals growth will be compensated until *veraison*. The dimensions of the canopy did not alter significantly over the three vintages when compared with CTR. Bud break and Potential Fertility indexes did not differ significantly from CTR. MED did not alter the porosity of the canopy, the number of leaf layers or the interior clusters (leaves percentage), - the removed leaf area was compensated until *veraison*. Ravaz index was significantly similar to CTR but the average value was substantially lower. MED vines displayed significantly lower incidence of *Botrytis*. The less compact clusters induced to lower disease incidence.

Grape composition and wine quality

MED grapevines produced grapes with similar Brix degree than CTR (Probable alcohol) and higher acidity. The berries are weightier and with more volume than the CTR.

The estimate Sugar Load for MED grapes was higher than CTR, which means that ripening occurs earlier. Adding to this, grape juice displayed less colour intensity, anthocyanins concentration and anthocyanins per berry are lower. It was also showed lower contents in total polyphenols. The better light exposure and cluster microclimate, combined with looser clusters and lower disease incidence in MED, was not enough for having improved grape quality. MED grapes showed to have higher content of carotenoids per berry; however, MED wines had lower concentrations of esters, alcohols, norisoprenoids, terpenes and sesquiterpenes.

Wine sensorial perception of quality

MED wines had higher overall score than CTR wines. MED wines showed to have descriptors like ‘Mushroom’, ‘Quince’, ‘Rose’, ‘Resin’, ‘Grapefruit’, ‘Dried fruits’, ‘Leather’ and ‘Tobacco’ and mentioned as perceptive and intense. MED wines benefit from having a lower *Botrytis* incidence and so they displayed higher sensorial scores and consumer acceptance (overall scores were similar to the Baga References).

These results indicated that lower yield might be obtained from MED and this technique, even though not showing improvement in ripening, colour or aromatic content, might be capable of improving consumer acceptance potential of Baga wines. MED requires machinery to be available at early times and it is not a time consuming technique.

The conclusions about the present research work referring to '*Double maturation raisonnée*' (DMR) are the following:

Yield components

DMR reduced significantly the grape production, by reducing significantly cluster and berry weight - it produced lighter and looser clusters (both significantly). Cluster and berry weight were significantly lower than CTR, cluster were significantly less compact. The effects of DMR on yield have been annually, overlapping the climate effects of the year. Cumulative effects were not found since there was not a significant variation of bud fertility the following years. DMR altered the distribution of the components of the Berry, skin with less weight and pulp with much lower weight, increasing the skin/pulp ratio when compared with CTR. DMR dehydration produced lighter berries. The number of seeds for DMR was similar to CTR.

Vegetative growth and health status

The dimensions of the canopy did not alter significantly over the three vintages when compared with CTR. Bud break and Potential Fertility indexes did not differ significantly from CTR. DMR did not alter the porosity of the canopy, the number of leaf layers or the interior leaves. The percentage of interior cluster was higher than CTR but, with the DMR intervention in the vine, around 85% of the clusters became exposed to sun light. Ravaz index was significantly similar to CTR but the values were always lower. DMR vines displayed lower incidence of *Botrytis*, but not significantly. The combination of more exposed clusters, extensive dryness and less compact clusters induced to lower disease incidence.

Grape composition and wine quality

DMR grapevines produced grapes with higher Brix degree than CTR (Probable alcohol) and higher acidity, resulting from concentration of pulp constituents. The berries have less weight and less volume. The estimate Sugar Load for DMR grapes was lower than CTR, which means that the ripening was in a lower advanced stage - the shoots were disconnected from the vine from 2 weeks prior to expected harvest date, so ripening should have stopped before harvest (this finding was confirmed with lower anthocyanin content per berry). Adding to this, grape juice displayed higher colour intensity, anthocyanins concentration, in total polyphenols and lower anthocyanins content per berry. Berry dehydration, higher light exposure and cluster microclimate, combined with looser clusters and lower disease incidence might be the reason of improved grape quality for DMR. DMR grapes showed to have lower content of carotenoids per berry, probably resulting from shoot disconnection from the vine. DMR wines displayed similar concentrations of esters, lower concentrations of alcohols, terpenes, norisoprenoids and sesquiterpenes.

Wine sensorial perception of quality

DMR wines had higher overall score than CTR wines. DMR wines showed to have descriptors like ‘Mushroom’, ‘Tobacco’, ‘Rose’, ‘Quince’, ‘Leather’ and ‘Tea’ mentioned as perceptive and intense. DMR wines benefit from having a lower *Botrytis* incidence and so they displayed higher sensorial scores and consumer acceptance, above the Baga wine References.

These results indicated that lower yield might be obtained from DMR, this technique might be capable of improving the oenological and consumer acceptance potential of Baga wines, resulting in overall scores above the Baga wine References. DMR is a labour time consuming technique.

The conclusions about the present research work referring to manual bunch thinning (MBT) are the following:

Yield components

MBT reduced significantly the grape production, by diminishing the number of clusters per vine. Cluster weight was lower than CTR, berry weight for MBT was also lower, and the cluster was less compact (all non-significantly). The effects of MBT on yield have been annually, overlapping by climate effects. There should not be cumulative effects since there was not a significant variation of bud fertility the following years. The fruit set ration was similar to CTR (significantly, although lower). MBT did not alter the distribution of the components of the Berry (pulp showed higher weight) but did not increase the skin/pulp ratio when compared with CTR. The number of seeds for MBT was higher than CTR, which can mean that only the more viable flowers were converted into berries.

Vegetative growth and health status

The dimensions of the MBT canopy did not alter significantly over the three vintages when compared with CTR. Bud break and Potential Fertility indexes did not differ significantly from CTR. MBT did not alter the number of leaf layers or the interior leaves percentage - the removed leaf area was compensated until *veraison*. The percentage of interior cluster and porosity of the canopy were significantly higher. Ravaz index was significantly similar to CTR but the values were always lower. MBT vines displayed significantly similar incidence of *Botrytis*.

Grape composition and quality

MBT grapevines produced grapes with similar Brix degree (Probable alcohol) and acidity than CTR. The berries had higher weight and volume.

The estimate Sugar Load for MBT grapes was higher than CTR, which means that the ripening was in a more advanced stage. Adding to this, grape juice displayed lower

colour intensity, anthocyanins concentration and higher anthocyanins per berry. It also showed to have higher content in total polyphenols. Although there was a gain in ripening with MBT (higher sugar load and anthocyanins per berry), apparently there was also a dilution effect that did not help to improve fruit quality (also combined with similar incidence of *Botrytis*). MBT grapes have a similar content of carotenoids per berry as CTR. MBT wines displayed lower concentrations of esters, alcohols, terpenes, norisoprenoids and sesquiterpenes. This may result in lower aromatic varietal notes.

Wine sensorial perception of quality

MBT wines had higher overall score than CTR wines. MBT wines showed to have descriptors like ‘Mushroom’, ‘Quince’, ‘Dried fruits’, ‘Resin’, ‘Rose’ and ‘Tobacco’ mentioned as perceptive and intense. MBT wines displayed higher sensorial scores and consumer acceptance than CTR, but lower than wines from all other studied modalities.

These results indicated that lower yield might be obtained from MBT; this technique may not be capable of improving the oenological and consumer acceptance potential of Baga wines. As MAD is a labour and time consuming technique, there should be careful considerate if there is some added-value in using MBT in these Baga vineyards and environmental conditions.

MAD, MED and DMR proved to be alternatives to MBT as techniques that achieved yield reduction and control. All three techniques improved grape and wine quality, conducting to better results that those obtained using MBT and CTR.

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Annexes

Annex 1 - Aromatic description of wines produced

Wine aroma compounds quantification was performed according to method described by Barros *et al*[1] at Laboratório deToxicologia, Departamento de Ciências Biológicas da Faculdade de Farmácia, Universidade do Porto (Porto, Portugal). The substances quantified by the method were: Ethyl butanoate, Ethyl hexanoate, Ethyl heptanoate, Ethyl octanoate, Ethyl decanoate, Ethyl dodecanoate, Ethyl 2-methyl-butanoate, Ethyl 3-methyl-butanoate, Ethyl *trans*-4-decenoate, Isoamyl acetate, Phenylethyl acetate, Diethyl succianate, Isoamyl hexanoate, Phenylethyl alcohol, Benzyl alcohol, α-Terpineol, β-*cis*-Terpineol, β - Linalol, Nerolidol, *Cis*-β-Farnesene, *trans*-α-Bisabolene, *trans*-Nerolidol, 4-Ethylguaiacol, Di-hydro pseudo-ionone, β-Damascenone, Limonene, Terpinolene, S-α-pinene, Geranyl acetone, Ethyl-2-hexenoate, Unidentified Sesquiterpene 1, Unidentified Sesquiterpene 2, Unidentified Sesquiterpene 3, Unidentified Sesquiterpene 4, Unidentified Terpene 1, Unidentifid Terpene 2, TDN, Neryl Acetate, Ethyl dl Malate, 4-Ethyl Phenol.

Table 1 shows the odour threshold limits for each quantified aromatic compound, odour descriptor and odorant series [2,3].

Table 1 - Compounds quantified by SPME-GC-MS method, odour descriptors, odorant series and odour threshold.

Compound	Odour threshold (µg/L)	Odour descriptors	Odorant series
ethyl butanoate (mg/l)	**20**	Papaya, pineapple, fruity, juicy, fruit.	1
ethyl hexanoate (mg/l)	**14**	Apple peel, pineapple, fruity, waxy, estery, green banana	1, 3, 4, 7
ethyl heptanoate (mg/l)	**22**	Fruity, pineapple, sweet, estery, banana, strawberry, cognac, green, spicy, oily	1, 3, 4, 5, 7
ethyl octanoate (mg/l)	**50 - 600**	Fruity, sweet, pear, pineapple, banana, apricot, fat, waxy, musty, wine, mushroom	1, 2, 3, 4, 6, 7
ethyl decanoate (mg/l)	**23 - 200**	Grape, apple, dry fruit, solvent, oily	1, 7
ethyl dodecanoate (mg/l)	**400 - 1500**	Sweet, waxy, soapy, rum, cream, floral	1, 2, 6, 7
ethyl 2-methyl-butanoate (A/10^7)	**18**	Apple, fruity, fruity, fresh, berry, grape, pineapple, mango, cherry	1, 3
ethyl 3-methyl-butanoate (A/10^7)	**3**	Apple, fruity, pineapple, green, orange, sipce	1, 3, 5
ethyl *trans*-4-decenoate (A/10^7)	not available	Green, fruity, oily, pineapple, apple, waxy	1, 3, 7
***iso* amyl acetate (mg/l)**	**30**	Banana, estery, apple	1, 3, 7
phenylethyl acetate (mg/l)	**250**	Rose, honey, Sweet, floral, yeasty, honey,cocoa, balsamic	2, 3, 4, 7
diethyl succianate (A/10^7)	**200000**	Cheese, earthy, spicy, cooked apple, ylang	2, 5, 6, 7
***iso* amyl hexanoate (A/10^7)**	**30**	Fruity, sweet, pineapple, pungent, sour cheese	1, 4, 6, 7
phenylethyl alcohol (mg/l)	**10000 - 14000**	Rose, lilac, bread, honey	2, 4, 7
benzyl alcohol (A/10^7)	**200000**	Flowery, sweet, rose, phenolic, balsamic	2, 3, 4, 7
α-terpineol (A/10^7)	**400**	Floral, citrus, sweet, pine, lilac, woody	1, 2, 3, 7
β-*cis*-terpineol (A/10^7)	**400**	Pungent, earthy, woody	3, 7
β - linalol (mg/l)	**25**	Muscat, citrus, fresh, floral, lavender, sweet, waxy	1, 2, 3, 4, 7
nerolidol (A/10^7)	**700 - 2250 - 10000**	Floral, green, citrus, woody, waxy	1, 2, 3, 7
limonene (µg/l)	**200**	Lemon, orange, citrus	1
terpinolene (A/10^7)	**0.2**	Citrus, Fresh, Pine, Plastic, Sweet, Woody	1, 3, 4, 7
s-α-pinene (A/10^7)	**0.006**	Pine, resin, turpentine	3, 7
geranyl acetone (µg/l)	**186**	Earth, Fatty, Floral, Fresh, Fruit, Green, Herbaceous, Magnolia, Meat, Nut, Rose, Spicy, Tropical, Wax, Wine	1, 2, 3, 5, 6, 7
neryl Acetate (µg/l)	**880 - 905400**	Floral, Rose, Fruity, Raspberry	1, 2
***cis*-β-farnesene (A/10^7)**	not available	Green apple, gardenia, green, citrus, woody	1, 2, 3, 7
***trans*-α-bisabolene (A/10^7)**	not available	Balsamic, oregano, citrus	1, 5, 7
***trans*-nerolidol (A/10^7)**	**700**	Green, floral, woody, fruity, citrus, melon	1, 2, 3, 7
4-ethylguaiacol (A/10^7)	**0.05 - 33**	Spicy, clove-like medicinal, woody, sweet vanilla, animal, barnyard, stable, phenolic, mousy	2, 5, 7
4-ethylphenol (A/10^7)	**440**	Smoke, savory, animal, barnyard, stable, phenolic, and mousy	2, 5, 7
dihydro pseudo-ionone (A/10^7)	not available	Sweet, waxy, citrus, floral, balsamic, dry, dusty, powdery, spicy	1, 2, 4, 5, 7
β-damascenone (µg/l)	**0.05**	Natural sweet, fruity, rose, plum, grape, raspberry, apple, honey, sugar, smoky, tobacco	1, 2, 4, 7
TDN (A/10^7)	**2**	Licorice, petrol	5, 7
ethyl-2-hexenoate (A/10^7)	**0.001**	Sweet, fruity, juicy, rum, green, vegetal	1, 3, 4, 7
ethyl malate (A/10^7)	not available	Caramel, sugar, brown sugar, sweet, wine, fruity, herbal	1, 3, 4, 7
unidentifid terpene 1 (A/10^7)	not available	non available	non available
unidentifid terpene 2 (A/10^7)	not available	non available	non available
unidentified sesquiterpene 1 (A/10^7)	not available	non available	non available
unidentified sesquiterpene 2 (A/10^7)	not available	non available	non available
unidentified sesquiterpene 3 (A/10^7)	not available	non available	non available
unidentified sesquiterpene 4 (A/10^7)	not available	non available	non available

1 = Fruity; 2 = Floral; 3 = Green, Fresh; 4 = Sweet; 5 = Spicy; 6 = Fatty; 7 = Others

Short chemical and sensorial description of quantified compounds

Below there is a small description about each quantified compound, regarding nomenclature and odour description, based in an online library, The Good Scents Company[4].

Ethyl Butanoate

Other names - Ethyl n-butanoate; Ethyl n-butyrate; Butanoic acid ethyl ester; Butyric acid ethyl ester; Butyric ether; UN 1180.

Odour type: fruity; Odour strength: high.

Odour description at 1.00 % in propylene glycol: fruity, juicy, fruit pineapple cognac.

Odour description: Sweet, fruity, tutti frutti, lifting and diffusive.

Taste description at 20.00 ppm: Fruity, sweet, tutti frutti, apple, fresh and lifting, ethereal.

Ethyl Hexanoate

Other names - Caproic acid ethyl ester; Ethyl butyl acetate; Ethyl n-hexanoate; Hexanoic acid ethyl ester; Hexanoic acid, ethyl ester; Hexanoic acid, monoethyl ester.

Odour type: fruity. Odour strength: high.

Odour description at 2.00 %: Sweet, fruity, pineapple, waxy, fatty and estery with a green banana nuance.

Taste description at 7.00 ppm: Sweet, pineapple, fruity, waxy and banana with a green, estery nuance.

Ethyl Heptanoate

IUPAC name - Ethyl heptanoate;

Other names - Ethyl enanthate; Ethyl heptylate, Heptanoic acid ethyl ester; Enanthic acid ethyl ester.

Odour type: fruity; Odour strength: medium

Odour description at 100.00 %: fruity pineapple cognac rum wine

Odour description: Fruity, pineapple, sweet, estery, banana, berry, cognac and slightly green with a seedy nuance.

Taste description at 20.00 ppm: Fruity, pineapple, banana and strawberry with a spicy, oily nuance.

Ethyl Octanoate

Other names - Ethyl caprylate; caprylic acid ethyl ester; ethyl caprylate; ethyl N-octanoate; ethyl octanoate (ethyl caprylate); ethyl octoate; ethyl octylate; ethylcaprylate; ethyloctanoate; octanoic acid ethyl ester;

Odour type: waxy; Odour strength: medium

Odour description at 100.00 %: fruity wine waxy sweet apricot banana brandy pear

Odour description: Waxy, sweet, musty, pineapple and fruity with a creamy, dairy nuance.

Taste description at 7.50 ppm: Sweet, waxy, fruity and pineapple with creamy, fatty, mushroom and cognac notes.

Ethyl Decanoate

Other names - capric acid ethyl ester; capric acid ethylester; capric acid, ethyl ester; decanoic acid ethyl ester; decanoic acid, ethyl ester; ethyl caprate; ethyl caprinate; ethyl decanoate; ethyl decylate; ethylcaprate.

Odour type: waxy. Odour strength: medium.

Odour description at 100.00 %: sweet waxy fruity apple grape oily brandy.

Odour description: Sweet, waxy, fruity, apple.

Taste description at 20.00 ppm: Waxy, fruity, sweet apple.

Ethyl Dodecanoate

Other names - dodecanoic acid ethyl ester; dodecanoic acid, ethyl ester; ethyl dodecanoate; ethyl dodecylate; ethyl laurate (ethyl dodecanoate); ethyl laurinate; ethyllaurate; lauric acid ethyl ester.

Odour type: waxy. Odour strength: medium.

Odour description at 100.00 %: sweet waxy floral soapy clean.

Odour description: Sweet, waxy, soapy and rummy with a creamy, floral nuance.

Taste description at 50.00 ppm: Waxy, soapy and floral with a creamy, dairy and fruity nuance.

Ethyl 2-methylbutanoate

Other names - berry butyrate; butanoic acid, 2-methyl-, ethyl ester; butyric acid, 2-methyl-, ethyl ester; dorintha; ethyl 2 methyl butyrate; ethyl 2 methyll butyrate synthetic; ethyl 2-methyl butanoate; ethyl 2-methylbutanoate; ethyl 2-methylbutyrate; ethyl a-methylbutyrate; ethyl alpha-methyl butyrate; ethyl DL-2-methylbutyrate; ethyl methyl butyrate-2; ethyl methyl butyrate-2 natural; ethyl methyl-2-butyrate; ethyl-2-methyl butyrate; 2-methyl butanoic acid ethyl ester; 2-methyl butyric acid ethyl ester; 2-

methylbutanoic acid ethyl ester; 2-methylbutyric acid ethyl ester; DL-2-methylbutyric acid ethyl ester.

Odour type: fruity. Odour strength: medium,

Odour description: Fruity, estery and berry with fresh tropical nuances.

Taste description at 10.00 ppm: Fruity, fresh, berry, grape, pineapple, mango and cherry notes.

Ethyl 3-methylbutanoate

Other names - butanoic acid, 3-methyl-, ethyl ester; butyric acid, 3-methyl-, ethyl ester; ethyl 3-methyl butanoate; ethyl 3-methyl butyrate; ethyl 3-methylbutanoate; ethyl 3-methylbutyrate; ethyl beta-methyl butyrate; ethyl iso valerate; ethyl isopentanoate; ethyl isovalerianate; ethylisovalerate; 3-methyl butanoic acid ethyl ester; 3-methyl butyric acid ethyl ester; 3-methylbutanoic acid ethyl ester; 3-methylbutyric acid ethyl ester; iso-valeric acid ethyl ester; iso-valeric acid ethylester; iso valeric acid, ethyl ester.

Odour type: fruity. Odour strength: high.

Odour description: Sweet, diffusive, estery, fruity, sharp, pineapple, apple, green and orange.

Taste description: at 30.00 ppm: Sweet, fruity, spice, metallic and green with a pineapple and apple lift.

Ethyl trans-4-decenoate

Other names - (4E)-4-decenoic acid ethyl ester; (E)-4- decenoic acid ethyl ester; trans-4-decenoic acid ethyl ester; 4-decenoic acid, ethyl ester, (4E)-; 4-decenoic acid, ethyl ester, (E)-; ethyl (4E)-dec-4-enoate; ethyl (E)-dec-4-enoate; ethyl 4-decenoate (trans); ethyl trans-4-decenoate; pear decenoate.

Odour type: green. Odour strength: medium.

Odour description at 100.00 %: green fruity waxy cognac.

Odour description: Green, fruity and oily with a pineapple, apple waxy nuance.

Taste description at 10.00 ppm: Fatty, waxy, green, pineapple and pear nuances.

Isoamyl acetate

Other names - acetic acid 3-methyl butyl ester; acetic acid 3-methylbutyl ester; acetic acid isoamyl ester; acetic acid isopentyl ester; acetic acid, 3-methylbutyl ester; acetic acid, isopentyl ester; iso-amyl acetate; butanol, 3-methyl-, acetate; 1-butanol, 3-methyl-, acetate; 3-methyl butyl acetate; beta-methyl butyl acetate; iso pentyl acetate; iso pentyl alcohol acetate; iso pentyl alcohol, acetate; iso pentyl ethanoate.

Odour type: fruity. Odour strength: high.

Odour description: Sweet, banana, fruity with a ripe estery nuance.

Phenylethyl acetate

Other names - acetic acid 2-phenyl ethyl ester; acetic acid 2-phenylethyl ester; acetic acid phenethyl ester; acetic acid, 2-phenylethyl ester; acetic acid, phenethyl ester; benzyl carbinyl acetate; benzylcarbinyl acetate; 2-phenethyl acetate; beta-phenethyl acetate; phenethyl alcohol acetate; phenethyl ethanoate; phenyl ethyl acetate; 2-phenyl ethyl acetate; beta-phenyl ethyl acetate; phenyl ethyl alcohol acetate; phenylethyl acetate; 2-phenylethyl acetate; 2-phenylethyl acetone.

Odour type: floral. Odour strength: medium.

Odour description at 100.00 %: floral, rose, sweet, honey, fruity, tropical.

Odour description at 100.00 %: Sweet, honey, floral rosy, with a slight yeasty honey note with a cocoa and balsamic nuance.

Taste description at 5.00 - 10.00 ppm: Sweet, honey, floral, rosy with a slight green nectar fruity body and mouth feel.

Diethyl succinate

Other names - butane dioic acid diethyl ester; butanedioic acid diethyl ester; butanedioic acid, di-C8-26-alkyl esters; butanedioic acid, diethyl ester; diethyl butane dioate; diethyl butane-1,4-dioate; diethyl butanedioate; diethyl ethane dicarboxylate; diethyl ethanedicarboxylate; diethyl succinate natural; diethyl succinate synthetic; ethyl succinate; succinic acid diethyl ester; succinic acid, diethyl ester.

Odour type: fruity. Odour strength: low.

Odour description at 100.00 %: mild, fruity, cooked apple, ylang.

Isoamyl hexanoate

Other names - iso amyl caproate; iso amyl hexanoate (caproate), natural; hexanoic acid 3-methyl butyl ester; hexanoic acid 3-methylbutyl ester; hexanoic acid isopentyl ester; hexanoic acid, 3-methylbutyl ester; hexanoic acid, isopentyl ester; 3-methyl butyl hexanoate; 3-methylbutyl hexanoate; iso pentyl alcohol hexanoate; iso pentyl alcohol, hexanoate; iso pentyl caproate; iso pentyl hexanoate; iso pentyl-N-hexanoate;

Odour type: fruity. Odour strength: medium.

Odour description at 100.00 %: fruity, banana, apple, pineapple, green.

Odour description: Fruity, sweet, pineapple with a slightly pungent sour cheesey note.

Taste Description at 25.00 ppm: Fruity, green, pineapple with a waxy nuance.

Phenylethanol

Other names - benzene ethanol; benzeneethanol; benzyl carbinol; benzyl methanol; 2-hydroxyethyl benzene; (2-hydroxyethyl)benzene; 2-hydroxyethylbenzene; b-hydroxyethylbenzene; mellol; beta-p.e.a.; b-phenethanol; 2-phenethyl alcohol; phenethylalcohol; phenethylol; 2-phenyl ethan-1-ol; phenyl ethanol; 2-phenyl ethanol; beta-phenyl ethanol; phenyl ethyl alcohol; 2-phenyl ethyl alcohol; beta-phenyl ethyl alcohol; 1-phenyl-2-ethanol; phenylethanol; 2-phenylethanol; b-phenylethanol; b-phenylethyl alcohol.

Odour type: floral. Odour strength: medium.

Odour description at 100.00 %: floral, rose, dried rose flower, rose water.

Odour description: Sweet, floral, fresh and bready with a rosey, honey nuance.

Taste description at 20.00 ppm: Floral, sweet, rosey and bready.

Benzyl alcohol

Other names - benzencarbinol; benzene carbinol; benzene methanol; benzenecarbinol; benzenemethanol; benzoyl alcohol; Benzylalcohol; benzylic alcohol; Benzylicum; (hydroxymethyl) benzene; (hydroxymethyl)benzene; hydroxytoluene; a-hydroxytoluene; alpha-hydroxytoluene; methanol, phenyl-; phenol carbinol; phenyl carbinolum; phenyl methanol; phenyl methyl alcohol; phenyl-methanol; phenylmethan-1-ol; phenylmethanol; phenylmethyl alcohol; a-toluenol; alpha-toluenol; ulesfia.

Odour type: floral. Odour strength: medium.

Odour description at 100.00 %: floral, rose, phenolic, balsamic.

Odour description: Sweet, floral, fruity with chemical nuances.

Taste description at 50.00 ppm: Chemical, fruity with balsamic nuances.

α Terpineol

Other names - 3-cyclohexene-1-methanol, a,a,4-trimethyl-; lindenol; p-menth-1-en-8-ol; para-menth-1-en-8-ol; 1-p-menthen-8-ol; 1-para-menthen-8-ol; 2-(4-methyl-1-cyclohex-3-enyl)propan-2-ol; 2-(4-methyl-3-cyclohexen-1-yl)-2-propanol; 2-(4-methyl-3-cyclohexenyl)-2-propanol; 1- methyl-4-isopropyl-1-cyclohexen-8-ol; 1-methyl-4-isopropyl-1-cyclohexene-8-ol; 2-(4-methyl-cyclohex-3-enyl)-propan-2-ol; 2-(4-methylcyclohex-3-en-1-yl) propan-2-ol; 2-(4-methylcyclohex-3-enyl) propan-2-ol; alpha-terpilenol; 1-α-terpineol; α-terpineol; DL-alpha-terpineol; alpha-terpineol (NQ) (natural); terpineol-alpha; terpineol alpha; tilianol NP; tilianol super; (1)-alpha,alpha,4-trimethyl cyclohex-3-ene-1-methanol; alpha,alpha,4- trimethyl-3-cyclohexene-1-methanol; (1)-alpha,alpha,4-trimethylcyclohex-3-ene-1-methanol.

Odour type: odourless. Odour strength: medium.

Odour description at 100.00 %: pine, terpene, lilac, citrus, woody, floral.

β-*cis*-Terpineol

Other names - cyclohexanol, 1-methyl-4-(1-methylethenyl)-; p-menth-8-en-1-ol; para-menth-8-en-1-ol; 1-methyl-4-(1-methyl ethenyl) cyclohexanol; 1-methyl-4-(1-methyl vinyl) cyclohexan-1-ol; 1-methyl-4-(1-methylethenyl)cyclohexanol; 1-methyl-4-(1-methylvinyl)cyclohexan-1-ol; 1-methyl-4-(prop-1-en-2-yl)cyclohexanol; 1-methyl-4-isopropenyl cyclohexan-1-ol; 1-methyl-4-isopropenylcyclohexan-1-ol; 1- methyl-4-ethyl vinyl cyclohexanol; 1- methyl-4-prop-1-en-2-ylcyclohexan-1-ol; 4-isopropenyl-1-methyl-1-cyclohexanol; 4-iso propenyl-1-methylcyclohexanol; terpin-1-ol.

Odour type: woody. Odour strength: medium.

Odour description at 10.00 % in dipropylene glycol: pungent, earthy, woody.

Linalol

Other names - coriander oil terpeneless; 3,7-dimethyl octa-1,6-dien-3-ol; 2,6-dimethyl octa-2,7-dien-6-ol; (+/-)-3,7-dimethyl-1,6-octadien-3-ol; (±)-3,7-dimethyl-1,6-octadien-3-ol; 3,7-dimethyl-1,6-octadien-3-ol; 2,6-dimethyl-2,7-octadiene-6-ol; (+/-)-3,7-dimethyl-3-hydroxy-1,6-octadiene; (±)-3,7-dimethyl-3-hydroxy-1,6-octadiene; 3,7-dimethyl-octa-1,6-dien-3-ol; 3,7-dimethylocta-1,6-dien-3-ol; 2,6-dimethylocta-2,7-dien-6-ol; linalool; linalol natural isolate; (±)-linalool; beta-linalool; p-linalool; para-linalool; linalyl alcohol; 1,6-octadien-3-ol, 3,7-dimethyl-.

Odour type: floral. Odour strength: medium.

Odour description at 100.00 %: citrus, floral, sweet, bois de rose, woody, green, blueberry.

Odour description: Citrus, orange, floral, terpenic, waxy and rose.

Taste description at 10.00 ppm: Citrus, orange, lemon, floral, waxy, aldehydic and woody.

Nerolidol

Other names - dodecatriene; 3-hydroxy-3,7,11-trimethyl-1,6,10-dodecatriene; Melaleucol; methyl vinyl homogeranyl carbinol; 3,7,11-trimethyl dodeca-1,6,10-trien-3-ol; 3,7,11-trimethyl-1,6,10-dodecatrien-3-ol; 3,7,11-trimethyldodeca-1,6,10-trien-3-ol.

Odour type: floral. Odour strength: low.

Odour description at 100.00 %: floral, green, waxy, citrus, woody.

Odour description: Floral, green and citrus like, with woody waxy nuances.

Taste description at 25.00 ppm: Green, floral, woody with fruity-citrus and melon nuances.

***Cis*-β-Farnesene**

Other names - (Z)-beta-farnesene; (6Z)-7,11-dimethyl-3-methylene-1,6,10-dodecatriene; (6Z)-7,11-dimethyl-3-methylidenedodeca-1,6,10-triene.

Odour Type: Green. Odour Strength: medium

Odour Description at 100.00 %: citrus, green.

***trans*-α-Bisabolene**

Other names - (9E)-bisabola-4,7(11),9-triene; trans-alpha-bisabolene; 4-[(1E)-1,5-dimethylhexa-1,4-dien-1-yl]-1-methylcyclohexene; 1-methyl-4-[(2E)-6-methylhepta-2,5-dien-2-yl]cyclohexene.

No organoleptic data was found.

***trans*-Nerolidol**

Other names - 1,6,10-dodecatrien-3-ol, 3,7,11-trimethyl-, (6E)-; 1,6,10-dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-; (±)-trans-nerolidol; (6E)-nerolidol; trans-nerolidol; nerolidol (E); nerolidol trans-form; trans-3,7,11-trimethyl dodeca-1,6,10-trien-3-ol; trans-trimethyl dodecatrien-3-ol; (6E)-3,7,11-trimethyl-1,6,10-dodecatrien-3-ol; (E)-3,7,11-trimethyl-1,6,10-dodecatrien-3-ol; trans-3,7,11-trimethyl-1,6,10-dodecatrien-3-ol; (E)-3,7,11-trimethyl-dodeca-1,6,10-trien-3-ol; (6E)-3,7,11-trimethyldodeca-1,6,10-trien-3-ol; (E)-3,7,11-trimethyldodeca-1,6,10-trien-3-ol

Odour Type: floral. Odour Strength: low.

Odour Description at 100.00 %: floral, green, citrus, woody, waxy.

Taste Description: green, floral, woody, fruity, citrus, melon.

4-Ethylguaiacol

Other names - homo creosol; 4-ethyl guaiacol; p-ethyl guaiacol; para-ethyl guaiacol; 4-ethyl-2-methoxyphenol; ethyl-4 guaiacol; 4-ethylguaiacol; p- ethylguaiacol; para-ethylguaiacol; guaiacol, 4-ethyl-; guaiacyl ethane; guaiacylethane; 1-hydroxy-2-methoxy-4-ethyl benzene; 1- hydroxy-2-methoxy-4-ethylbenzene; 4-hydroxy-3-methoxy ethylbenzene; 4-hydroxy-3-methoxyethyl benzene; 4-hydroxy-3-methoxyphenyl ethane; 4-hydroxy-3-methoxyphenylethane; 2-methoxy-4-ethyl phenol; 2-methoxy-4-ethylphenol; phenol, 4-ethyl-2-methoxy-

Odor type: spicy. Odor strength: medium.

Odor Description: Spicy and clove-like with medicinal, woody and sweet vanilla nuances.

Taste Description at 30.00 ppm: Woody, smokey and spicy with a sweet vanilla background.

Di-hydro pseudo-ionone

No information found.

β-Damascenone

Other names - 2- buten-1-one, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-, (2E)-

2- buten-1-one, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-, (E)-; damascenone; beta-damascenone; fermentone; floriffone; roastarome natural; rose ketone-4; rosenone; 4-(2,6,6-trimethyl cyclohexa-1,3-dienyl) but-2-en-4-one; trimethyl cyclohexadienyl butanone; trans-2,6,6-trimethyl-1-(2-butenoyl)cyclohexa-1,3-diene; (E)-1-(2,6,6-trimethyl-1-cyclohexa-1,3-dienyl)but-2-en-1-one; 2,6,6-trimethyl-1-trans-crotonoyl-1,3-cyclohexadiene; (2,6,6-trimethyl-1,3-cyclohexadien-1-yl) butanone; (2E)-1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one; (E)-1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one; 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one; (2E)-1-(2,6,6-trimethylcyclohexa-1,3-dien-1-yl)but-2-en-1-one

Odor type: floral. Odor strength: high.

Odor Description: Woody, sweet, fruity, earthy with green floral nuances.

Taste Description at 20.00 ppm: Woody, floral, herbal, green and fruity with spicy tobacco nuances.

Limonene

Other names - acintene dp dipentene; cajeputene; cinene; citrene; dipanol; dipentene; (±)-dipentene; eulimen; limonene; (±)-alpha- limonene; dextro,laevo-limonene; DL- limonene; limonene X; (±)- limonene; p-mentha-1,8-diene; para-mentha-1,8-diene; dextro,laevo-para-mentha-1.8-diene; DL-p-mentha-1.8-diene; 1,8(9)-p-menthadiene; 1,8(9)-para-menthadiene; 1-methyl-4-(1-methyl ethenyl) cyclohexene; 1-methyl-4-(1-methyl vinyl) cyclohexene; 1-methyl-4-isopropenyl-1-cyclohexene; 1-methyl-4-prop-1-en-2-ylcyclohexene; orange tetrarome; orange tetrarome # 987431; 4-isopropenyl-1-methyl-1-cyclohexene; delta-1,8-terpodiene.

Odor Type: citrus. Odor Strength: medium.

Odor Description at 100.00 %: citrus, herbal, terpene, camphor.

Terpinolene

Other names - cyclohexene, 1-methyl-4-(1-methylethylidene)-; cyclohexene, 3-methyl-6-(1-methylethylidene)-; p- menth-1,4,8-diene; para-menth-1,4,8-diene; p-menth-1,4(8)-diene; p-mentha-1,4,8-diene; para-mentha-1,4,8-diene; p-mentha-1,4(8)-diene; para-mentha-1,4(8)-diene; 1,4(8)-p-menthadiene; 1,4(8)-para-menthadiene; p-meth-1-en-8-yl-formate; 1-methyl-4-(1-methyl ethylidene) cyclohexene; 1-methyl-4-(1-methylethylidene)-1-cyclohexene; 1-methyl-4-(1-methylethylidene)cyclohexene; 1-methyl-4-(propan-2-ylidene)cyclohexene; 1-methyl-4-isopropylidene-1-cyclohexene; 1-methyl-4-propan-2-ylidenecyclohexene; 4-iso propylidene-1-methyl cyclohexene; 4-iso propylidene-1 methylcyclohexene; 4-iso propylidene-1-methylcylohexene; 1,4,8-terpadiene; 1,4(8)-terpadiene; iso terpinene; terpineolene.

Odor Type: herbal. Odor Strength: medium.

Odor Description at 1.00 %: Sweet, fresh, piney, citrus with a woody old lemon peel nuance.

Taste Description at 2.00 - 25.00 ppm: Woody, terpy, lemon and lime-like with a slight herbal and floral nuance.

S-α-pinene

Other names - (-)- pin-2(3)-ene; (-)-a-pinene; (1S,5S)-2-pinene; (1S)-(-)-alpha-pinene; L-(-)-alpha-pinene; L-alpha- pinene; laevo-alpha- pinene; alpha-pinene L-, natural; alpha-pinene laevo natural; alpha- pinene laevo-; (1S,5S)-2,6,6-trimethyl bicyclo(3.1.1)hept-2-ene; (1S,5S)-4,7,7-trimethylbicyclo[3.1.1]hept-3-ene.

Odor Type: terpenic. Odor Strength: medium.

Odor Description at 10.00 % in dipropylene glycol: sharp, warm, resinous, fresh, pine.

Geranyl acetone

Other names - 6,10-dimethyl undeca-5,9-dien-2-one; 6,10-dimethyl-5,9-undecadien-2-one; 6,10-dimethyl-undeca-5,9-dien-2-one; 6,10-dimethylundeca-5,9-dien-2-one; 5,9-undecadien-2-one, 6,10-dimethyl-.

Odor Type: floral. Odor Strength: medium.

Odor Description at 100.00 %: fresh, rose, leaf, floral, green, magnolia, aldehydic, fruity.

Taste Description: floral, rose, fresh, soapy.

Ethyl-2-hexenoate

Other names - ethyl hex-2-enoate; hex-2-enoic acid ethyl ester; 2-hexenoic acid ethyl ester; 2- hexenoic acid, ethyl ester; 2- hexenoic acid, ethylester.

Odor Type: fruity. Odor Strength: medium.

Odor Description at 100.00 %: rum, fruity, green, sweet, juicy.

TDN

Other names - 1,2-dihydro-1,1,6-trimethyl naphthalene; 1,2-dihydro-1,1,6-trimethylnaphthalene; naphthalene, 1,2-dihydro-1,1,6-trimethyl-; 1,1,6-trimethyl-1,2-dihydronaphthalene; 1,1,6-trimethyl-2H-naphthalene

Odor Type: licorice. Odor Strength: medium.

Odor Description at 10.00 % in dipropylene glycol: Licorice.

Neryl Acetate

Other names - acetic acid neryl ester; cis-3,7-dimethyl-2,6-octadien-1-ol acetate; (Z)-3,7-dimethyl-2,6-octadien-1-yl acetate; cis-3,7- dimethyl-2,6-octadien-1-yl acetate; (Z)-3,7-dimethyl-2,6-octadien-1-yl ethanoate; cis-3,7-dimethyl-2,6-octadien-1-yl ethanoate; (2Z)-3,7-dimethylocta-2,6-dien-1-yl acetate; (Z)-3,7- dimethylocta-2,6-dien-1-yl acetate; [(2Z)-3,7-dimethylocta-2,6-dienyl] acetate; (Z)- geranyl acetate; nerol acetate; neryl /geranyl acetate natural; (Z)- neryl acetate; cis-neryl acetate; neryl acetate pure; neryl ethanoate; 2,6- octadien-1-ol, 3,7-dimethyl-, acetate, (2Z)-; 2,6-octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-.

Odor Type: floral; Odor Strength: medium

Odor Description at 100.00 %: floral, rose, soapy, citrus, dewy, pear.

Ethyl dl Malate

Other names - butanedioic acid, 2-hydroxy-, diethyl ester; butanedioic acid, hydroxy-, diethyl ester; butanedioic acid, hydroxy-, diethyl ester, (±)-; diethyl 2-hydroxy-1,4-butane dioate; diethyl 2-hydroxybutanedioate; diethyl DL-malate; diethyl hydroxybutane dioate; diethyl hydroxybutanedioate; diethyl-2-hydroxybutandioate; ethyl malate; hydroxybutane dioic acid diethyl ester; malic acid diethyl ester; malic acid, diethyl ester.

Odor Type: caramellic. Odor Strength: medium.

Odor Description at 100.00 %: brown sugar, sweet, wine, fruity, herbal.

4-Ethyl Phenol

Other names - benzene,1-ethyl,4-hydroxy; p-ethyl phenol; para-ethyl phenol; 1-ethyl-4-hydroxybenzene; 4-ethylphenol; p-ethylphenol; para-ethylphenol; 1-hydroxy-4-ethyl benzene; 1-hydroxy-4-ethylbenzene; 4-hydroxyethyl benzene; 4-

hydroxyethylbenzene; 4-hydroxyphenyl ethane; (4-hydroxyphenyl)ethane; 4-hydroxyphenylethane; phenol, 4-ethyl-; phenol, p-ethyl-.

Odor Type: smoky. Odor Strength: high.

Odor Description at 1.00 %: Smoke, phenolic, creosote and savory.

Taste Description at 2.50 ppm: Phenolic, smoke, bacon and ham

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[1] Barros EP, Moreira N, Pereira GE, Leite SGF, Rezende CM, Guedes de Pinho P. Development and validation of automatic HS-SPME with a gas chromatography-ion trap/mass spectrometry method for analysis of volaties in wines. Talanta 2012; 101: 177-186.

[2] Xie K, Feng T, Lin W, Zhuang H, Xu Z, Bing F. Correlations between aroma profiles and sensory characteristics of red wines by using Partial Least Squares regression method. Advance Journal of Food Science and Technology. 2016. 12(5): 271-280.

[3] Tao Y, Zhang L. Intensity prediction of typical aroma characters of cabernet sauvignon wine in Changli County (China). LWT - Food Science and Technology. (2010). 431550-1556.

[4] Consulted online at December 2015; http://www.thegoodscentscompany.com

Annex 2 - OIV204 - Cluster compactness

Carattere: Caractère: Merkmal: Characteristic: Carácter:	Grappolo: compattezza Grappe: compacité Traube: Dichte Bunch: density Racimo: compacidad	Codes N^{os} OIV 204 UPOV 33 IPGRI 6.2.3

Livelli di espressione / Notation / Bonitierung / Notes / Notación:

1	3	5	7	9
molto spargolo	spargolo	medio	compatto	molto compatto
très lâche	lâche	moyenne	compacte	très compacte
sehr locker	locker	mittel	dicht	sehr dicht
very loose	loose	medium	dense	very dense
muy suelto	suelto	medio	compacto	muy compacto

Varietà di riferimento / Exemples de variétés / Beispielssorten / Example varieties / Ejemplos de variedades:

1	3	5	7	9
V. amurensis	Perle von Csaba B	Chasselas B	Barbera N	Meunier N
Uva rara N	Cardinal Rg	Schiava Grossa N	Sauvignon B	Silvaner B
	Prosecco B		Chenin B	
	Vermentino B			

Indicazioni / Définitions / Definitionen / Definitions / Indicaciones:

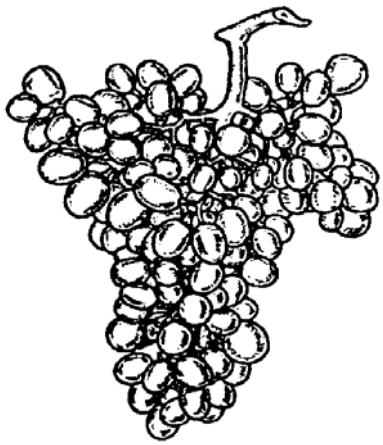
I: Osservazione da effettuare a maturità. Rilievo sui grappoli più grandi di 10 germogli. 1 = acini nettamente staccati, molti pedicelli visibili; 3 = acini appena staccati l'uno dall'altro, qualche pedicello visibile; 5 = acini appressati, pedicelli non visibili, acini che si possono muovere; 7 = acini che non si possono muovere direttamente; 9 = acini deformati dalla compressione.

F: Observation à faire à la maturité. Notation sur les plus grandes grappes de 10 rameaux. 1 = baies nettement séparées, nombreux pédicelles visibles; 3 = baies séparées les unes des autres, quelques pédicelles visibles; 5 = baies serrées, pédicelles non visibles, baies peuvent bouger; 7 = baies ne peuvent pas bouger directement; 9 = baies déformées par la pression.

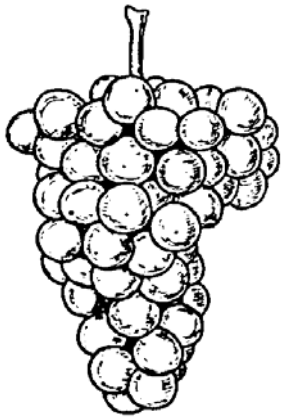
D: Feststellung bei der Reife. Beurteilung der größten Trauben von 10 Trieben. 1 = Beeren deutlich getrennt, viele sichtbare Beerenstielchen; 3 = Beeren lose miteinander verbunden mit einigen sichtbaren Beerenstielchen; 5 = dicht verteilte Beeren, Beerenstielchen nicht sichtbar, Beeren beweglich; 7 = Beeren nicht frei beweglich; 9 = Beeren durch Druck deformiert.

E: Observation at maturity. Examination of the largest bunches of 10 shoots. 1 = berries clearly separated, many visible pedicels; 3 = berries in loose contact with each other with some visible pedicels; 5 = densely distributed berries, pedicels not visible, berries are movable; 7 = berries not readily movable; 9 = berries deformed by compression.

S: Observación a realizar en racimos maduros. Notación de los racimos mayores de 10 sarmientos. 1 = bayas muy sueltas, con muchos pedicelos visibles; 3 = bayas separadas unas de otras, con algunos pedicelos visibles; 5 = bayas apretadas con pedicelos no visibles, bayas movibles; 7 = bayas dificilmente movibles; 9 = bayas deformadas por la presión.



3



7

Annex 3 - Score tasting table for Baga

			Grau de intensidade																													
			CTR						MAD						MED						MBT						DMR					
Família de aromas		**Aroma**	**0**	**1**	**2**	**3**	**4**	**5**	**0**	**1**	**2**	**3**	**4**	**5**	**0**	**1**	**2**	**3**	**4**	**5**	**0**	**1**	**2**	**3**	**4**	**5**	**0**	**1**	**2**	**3**	**4**	**5**
Floral	**Floral**	**Rosa**																														
		Violeta																														
		Cravo																														
Frutado	**Fruta verde**	**Maça**																														
		Pera																														
		Marmelo																														
	Citrinos	**Tangerina**																														
		Toranja																														
	Frutos tropicais	**Banana**																														
		Ananás																														
	Frutos vermelhos	**Groselha**																														
		Framboesa																														
		Cereja																														
		Ameixa																														
		Amora																														
	Fruta seca	**Passa ameixa**																														
		Compota																														
Frutos secos	**Frutos secos**	**Noz**																														
		Avelã																														
		Amêndoa																														
Herbáceo	**Herbáceo seco**	**Chá**																														
		Tabaco																														
	Herbáceo fresco	**Pimento**																														
		Ervas																														
		Resina																														
Especiarias	**Picante**	**Pimenta**																														
		Alcaçuz																														
	Doce	**Cravinho**																														
Madeira	**Madeira**	**Baunilha**																														
		Tostado																														
		Cedro																														
		Carvalho																														
		Fumo																														
Animal	**Animal**	**Couro**																														
Fermentação	**Autolíticos**	**Pão**																														
		Levedura																														
	FML	**Natas**																														
		Manteiga																														
		Iogurte																														
		Metálico																														
Maturação	**Maturação**	**Mel**																														
		Tabaco																														
		Café																														
		Chocolate																														
		Cogumelo																														
Gordura	**Gordura**	**Ranço/Queijo**																														
			0 (nada); 1 (muito pouco); 2 (pouco); 3 (notório); 4 (intenso); 5 (muito intenso)																													

Annex 4 - Baga description

Segue-se um pequeno resumo das características varietais da Baga (*Vitis Vinifera L.*) tendo por base alguma documentação publicada anteriormente [i,ii].

Origem da casta: Aguiar (1867) e Vila Maior (1875) limitam a classificação da casta à Bairrada.
Região de maior expansão: Ocupa mais de metade da superfície vitícola bairradina.
Sinónimos oficiais (nacional e OIV): Bagrina Crvena (YU).
Sinónimos históricos e regionais: Paga Dívida (Dão), Poeirinho (Ribatejo, Cantanhede, Coimbra), Tinta da Bairrada (Douro), Carrasquenho (Tomar), Baga de Louro (Dão e Bairrada).
Homónimos: Desconhecidos.
Superfície vitícola actual: 9.200 ha.
Utilização actual a nível nacional: 0,6%
Tendência de desenvolvimento: Decrescente.
Intravariabilidade varietal da produção: Intermédia.
Qualidade do material vegetativo: Material policlonal RNSV. Material clonal RNSV em processo de admissão à certificação.

VVMD5		VVMD7		VVMD27		VrZag62		VrZag79		VVS2	
Alelo1	Alelo2	Alelo1	Alelo2	Alelo1	Alelo2	Alelo1	Alelo2	Alelo1	Alelo2	Alelo1	Alelo2
232	240	235	235	179	189	188	204	247	251	145	157

Classificação Regional
Vinho de Qualidade DOC: «Douro», «Bairrada», «Beira interior», «Alenquer», «Ribatejo», sub-regiões de Almeirim, Cartaxo, Chamusca, Tomar;
Vinho de qualidade IPR: «Encosta de Aire», «Alcobaça».
Vinho regional: «Minho», «Beiras» em todas as sub-regiões, «Estremadura»,«Ribatejano», «Alentejano», «Algarve».

Morfologia
Extremidade do ramo jovem: Aberta, com forte densidade de pêlos prostrados e orla carmim fraca.
Folha jovem: Verde com placas bronzeadas, página inferior com forte densidade de pêlos prostrados.
Flor: Hermafrodita.
Pâmpano: Estriado de vermelho, média intensidade antociânica dos gomos.
Folha adulta: Tamanho médio, pentagonal, com cinco lóbulos; limbo verde-médio a escuro, ligeiramente revoluto, bolhosidade fraca, página inferior com forte densidade de pêlos prostrados; dentes curtos e convexos; seio peciolar pouco aberto, com a base em V, seios laterais fechados em U.
Cacho: Médio, cónico, compacto, pedúnculo de comprimento médio.
Bago: Arredondado, médio e negro-azul; película de espessura média, polpa mole.
Sarmento: Castanho-escuro.

Fenologia
Abrolhamento: Época média, 10 dias após a Castelão.
Floração: Época média, 6 dias após a Castelão.
Pintor: Época média, 2 dias após a Castelão.
Maturação: Tardia, duas semanas após a Castelão.

Potencial Vegetativo
Vigor: Médio-forte.
Porte (tropia): Semi-erecto, algumas varas prostradas e retombantes.
Entrenós: Médios.
Tendência para o desenvolvimento de netas: Forte.
Rebentação múltipla: Pouca.
Índice de fertilidade: Elevado.
Produtividade: Medianamente produtivo (até 15.000 l/ha). Valores RNSV: 2,2 kg/pl (média de, no mínimo, 40 cultivares, registada em Anadia, durante 5 anos).
Estabilidade da produção (diferentes anos e localidades): Estável.
Homogeneidade de produção (entre as plantas): Uniforme.
Índice de Winkler (somatório de temperaturas activas): Elevado. Por isso, surgem problemas na maturação com as chuvas de Setembro.
Producção recomendada: 5.000 l/ha.
Sensibilidade abiótica: Geralmente apresentam bom comportamento.
Sensibilidade criptogâmica: Pouco sensível ao Míldio e ao Oídio, é muito sensível à podridão de cachos.
Estado sanitário (sistémico) antes da selecção: 45% GLRaV 3, <50% GFkV.
Sensibilidade a parasitas: Medianamente sensível à Cigarrinha Verde.
Tamanho do cacho: 260 g em média, mas heterogéneo.
Compactação do cacho: Muito compacto.
Bago: Médio (1,5 g).
Película: Média espessura, delicada.
Nº de graínhas: 2,6 por bago.

Potencial Agronómico
Sistema de condução: Alta, cordão bilateral, forma tradicional guyot múltiplo.
Solo favorável para obter qualidade: Adapta-se a todos os tipos de solo, mas recomenda-se terrenos de média fertilidade e humidade, bem drenados, com limitada disponibilidade hídrica; são favoráveis zonas argilo-calcárias jurássicas, mas dá-se mal com pH baixo.
Clima favorável: De intensa insolação e de Verão prolongado.
Compasso: Adapta-se a todos os intervalos que consideram o vigor desta casta.
Porta-enxertos: Boa afinidade com todos os porta-enxertos. Para obter boa qualidade do vinho, especialmente em solos férteis devem ser utilizados porta-enxertos de reduzido vigor.
Desavinho/Bagoinha: Não susceptível.
Conservação do cacho após maturação: Baixa.
Protecção contra ataques de pássaros: Reduzida.
Aptidão para vindima mecânica: Boa.

Potencial Enológico
Tipo de vinho: Vinho de qualidade, espumante tinto e vinho rosado.
Grau alcoólico provável do mosto: Médio. Devido à elevada fertilidade, a casta não consegue amadurecer em solos férteis e húmidos. Consegue-se muito boa qualidade desta casta, através do controlo da produção. Valores RNSV: 10,35% vol. (média de, no mínimo, 40 cultivares, registada em Anadia, durante 7 anos).
Acidez natural: Relativamente elevada (6-7 g/l). Valores RNSV: 5,88 g/l (média de, no mínimo, 40 cultivares, registada em Anadia, durante 7 anos).
Autocianinas totais: Valores RNSV: 865,86 mg/l (média de, no mínimo, 40 cultivares, registada em Anadia, durante 2 anos).
Índice de polifenóis totais (280nm) do mosto: Valores RNSV: 30,52 (média de, no mínimo, 40 cultivares, registada em Anadia, durante 2 anos).
Sensibilidade do mosto à oxidação: Apenas quando o estado sanitário das uvas é impróprio.

Intensidade da cor: Elevada.
Tonalidade: Rubi granada.
Sensibilidade do vinho à oxidação: Muito estável.
Análise laboratorial dos aromas: Presença de elevado número de compostos terpenóides, sesquinóides e norisoterpenóides.
Capacidade de envelhecimento do vinho: Excelente. Boa aptidão para envelhecimento em madeira.
Recomendação para lote: Touriga Nacional, Syrah.
Potencial para vinho elementar: Possível, originando vinhos equilibrados.
Caracterização habitual do vinho: Os vinhos apresentam cor intensa, rubi ou granada, por vezes com tons violáceos. O aroma é muito frutado, com notas de amora, compota, mel e cânfora. Na boca, os vinhos de Baga jovem são, frequentemente, um pouco delgados, com taninos fortes e pouco cobertos. Com uma maturação correcta, os taninos arredondam e os vinhos ganham volume e persistência (Cardoso, 2005).
Qualidade do vinho: Casta muito polémica devido à sua extrema capacidade produtiva. Em condições adequadas fornece dos melhores vinhos do país.

Particularidade da casta: A folha adulta aparece muitas vezes com um «dente» num dos seios laterais superiores. Cacho pequeno, compacto e alado, porte retombante. Vinho muito taninoso, mas de elevada frescura e longevidade.

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[i] Böhm J. Portugal Vitícola. O Grande Livro das Castas. 1ª Edição. 2007. Chaves Ferreira-Publicações SA. Lisboa.
[ii] IVV, Faustino R (Coordenador), Castro R (Coordenador Técnico-Científico). Catálogo das Castas Para Vinho Cultivadas Em Portugal. Volume 1 - 1ª Edição. 2011. Chaves Ferreira-Publicações SA. Lisboa.