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Nawel Benbouguerra

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**THÈSE POUR OBTENIR LE GRADE DE
DOCTEUR DE L'UNIVERSITÉ DE
MONTPELLIER**
En Sciences Alimentaires

**École doctorale GAIA – Biodiversité, Agriculture, Alimentation,
Environnement, Terre, Eau**

Unité de recherche : UMR Sciences Pour l'œnologie

**Évolutions structurales et propriétés biologiques des polyphénols
au cours de la maturation des baies de *vitis vinifera***

Présentée par Nawel Benbouguerra

Le 30 octobre 2020

Sous la direction de M. Cédric Saucier et M. Tristan Richard

Devant le jury composé de

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**UNIVERSITÉ
DE MONTPELLIER**

Dédicaces

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Table des matières

Dédicaces.....	2
Résumé	7
Abstract	8
Remerciements	9
Valorisation des travaux de recherche :	11
Liste des figures	12
Liste des tableaux.....	13
Liste des abréviations.....	14
Introduction générale.....	17
Chapitre 1 : Bibliographie	20
1. Le raisin au cours de la maturation.....	21
1.1. Le raisin	21
1.2. Le développement de la baie du raisin.....	23
2. Les polyphénols du raisin.....	25
2.1. Biosynthèse générale, familles de composés (flavonoïdes/non-flavonoïdes)	26
2.1.1. Les flavonoïdes	28
2.1.1.1. Les flavanols	28
2.1.1.2. Les anthocyanes	29
2.1.1.3. Les flavanones	30
2.1.1.4. Les flavonols	31
2.1.1.5. Les flavones	31
2.1.1.6. Les isoflavones.....	32
2.1.2. Les non- flavonoïdes	32

2.1.2.1. Les stilbènes	32
2.1.2.2. Les acides phénols.....	34
2.2. Accumulation et concentration en composés phénoliques au cours de la maturation du raisin rouge.....	35
2.2.1. Les polyphénols totaux	35
2.2.2. Les flavanols.....	35
2.2.3. Les anthocyanes	36
2.2.4. Les stilbènes.....	37
2.2.5. Les flavonols.....	37
3. Les activités biologiques du raisin	42
3.1. L'activité antioxydante	42
3.1.1. Espèces réactives oxygénées et stress oxydant	42
3.1.2. Antioxydants	44
3.1.3. Détermination de la capacité antioxydante	47
3.1.3.1. Tests spectrophotométriques	47
3.1.3.2. Tests électrochimiques.....	50
3.2. L'activité anti-inflammatoire	50
3.2.1. Inflammation	50
3.2.2. Marqueurs de l'inflammation.....	51
3.2.3. Complications de l'inflammation	52
3.3. L'activité antidiabétique	53
3.3.1. Physiopathologie du diabète.....	53
3.3.2. Diagnostiques, symptômes et complications du diabète	56
3.3.3. Traitements pharmacologiques	58
3.3.4. Traitements par les polyphénols.....	60
References	61
Chapitre 2: Les stilbènes du raisin et du vin et leurs propriétés anti-obésité.....	88

Background	90
Scope and Approach	90
Key Findings and Conclusions	90
Abbreviation list	91
Highlights.....	95
Introduction.....	95
Stilbenes content in grape berries and wine	104
Grape berries stilbenes	104
Stage of ripening	107
Grape varieties and species	107
External stimuli	108
Wine stilbenes.....	109
Duration of maceration	112
Yeast activities.....	112
Winemaking processes and wine ageing	113
Analytical methods for stilbenes analysis in grapes and wines	113
Capillary electrophoresis	115
Gas chromatography.....	115
Liquid Chromatography.....	115
Stilbenes, in vitro and in vivo anti-obesity effects and molecular mechanisms	120
Anti-obesity in vitro effects of stilbenes	122
Anti-obesity in vivo effects of stilbenes.....	136
Conclusion.....	149
References	151
Chapitre 3: Propriétés antioxydantes des extraits de pépins et de pellicules de trois cépages rouges au cours de la maturité.....	170

Chapitre 4: Propriétés antioxydantes et anti-inflammatoires des extraits de pellicules de 3 cépages rouges au cours de la maturation.....	192
Chapitre 5: L'inhibition de l'enzyme alpha-glucosidase par des extraits de pépins et de pellicules de Tannat au cours de la maturité	222
Conclusion générale.....	243
Perspectives	255
Résumé	256
Abstract	257

Résumé

Les polyphénols appartiennent à la famille des métabolites secondaires présents dans les plantes et majoritairement dans les baies de raisin. Ils jouent un rôle important dans la protection de la plante contre les stress biotiques et abiotiques. Ils ont un impact sur la qualité organoleptique des certains aliments comme ceux provenant du raisin et sont connus majoritairement pour leurs rôles bénéfiques pour la santé humaine. Une étude globale sur la composition phénolique et les activités biologiques (antioxydante, anti-inflammatoire et anti-diabète) d'extraits phénoliques de pépins et pellicules de trois cépages rouges Merlot, Tannat et Syrah à différents stades de maturité a été réalisée durant cette thèse. Les extraits de pépins ont une teneur plus élevée en composés phénoliques que les extraits de pellicules pour tous les stades de maturité. La composition phénolique diffère selon le stade de maturité et le tissu de la baie de raisin (pépin ou pellicule). L'activité antioxydante des extraits a été déterminée par des tests spectrophotométriques (DPPH, ABTS et FRAP) et des paramètres électrochimiques. L'activité anti-inflammatoire a été suivie par l'inhibition de la production des EROs et NO par les cellules RAW 264.7. L'activité anti-diabétique a été déterminée par la mesure d'inhibition de l'enzyme α -glucosidase. Les activités biologiques sont positivement corrélées avec la teneur en flavanols et négativement corrélées avec la teneur en anthocyanes et stilbènes. Les activités biologiques les plus importantes ont été mesurées avant la maturité pour tous les tests et dans les trois cépages étudiés.

Abstract

Polyphenols belong to the family of secondary metabolites found in plants and mainly in grape berries. They play an important role in the protection of the plant against biotic and abiotic stresses. They have an impact on the organoleptic quality of certain foods such as those from grapes and are mainly known for their beneficial roles for human health. A global study on the phenolic composition and the biological activities (antioxidant, anti-inflammatory and anti-diabetes) of the phenolic extracts of the seeds and skins of three red grape varieties Merlot, Tannat and Syrah at different stages of maturation was carried out during this thesis. Seed extracts contain a higher content of phenolic compounds than skin extracts for all stages of ripening. The phenolic composition differs depending on the stage of maturity and the tissue of the grape berry (seed or skin). The antioxidant activity of the extracts was determined by spectrophotometric tests (DPPH, ABTS and FRAP) and electrochemical parameters. Anti-inflammatory activity was followed by the inhibition of ROS and NO production by stimulated RAW 264.7 cells. Anti-diabetic effect was determined by measuring the inhibition of the enzyme α -glucosidase activity. The biological activities are positively correlated with the content of flavanols and negatively correlated with the content of anthocyanins and stilbenes. The most important biological activities were measured before maturity for all the tests and in the three grape varieties studied.

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Valorisation des travaux de recherche :

Publications

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2. Dudoit, A., Benbougerra, N., Richard, T., Hornedo-Ortega, R., Valls-Fonayet, J., Coussot, G., & Saucier, C. (2020). α -Glucosidase Inhibitory Activity of Tannat Grape Phenolic Extracts in Relation to Their Ripening Stages. *Biomolecules*, 10(8), 1088.
3. Nawel Benbougerra, Ruth Hornedo-Ortega, François Garcia, Toni El Khawand, Cédric Saucier, et Tristan Richard. 2020. « Stilbenes in grape berries and wine and their potential role as anti-obesity agents: a review », 63. Soumis (Avril 2020) dans le journal Trends in Food and Technology.
4. Nawel Benbougerra, Ruth Hornedo-Ortega, Josep Valls-Fonayet, Stéphanie Krisa, François Garcia, Cédric Saucier et Tristan Richard. 2020. « Polyphenolic characterization of Merlot, Tannat and Syrah skin extracts at different degrees of maturity and anti-inflammatory potential in RAW 264.7 cells », 19. Soumis (Septembre 2020) dans le journal Agricultural and Food Chemistry.

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Liste des figures

Figure 1. Production mondiale du raisin en 2018.....	21
Figure 2. Structure d'une baie du raisin	22
Figure 3. Diagramme montrant la taille et la couleur relative des baies au cours de la maturation.....	24
Figure 4. La voie biosynthétique des principaux composés phénoliques du raisin.	27
Figure 5. Structure des principaux flavonoïdes.....	28
Figure 6. Les monomères de flavanols présents dans le raisin	29
Figure 7. Structures des anthocyanes les plus présentes dans le raisin	30
Figure 8. Structures des flavanones présents dans le raisin	30
Figure 9. Structures des flavonols présents dans le raisin.....	31
Figure 10. Structures des flavones présents dans le raisin	32
Figure 11. Structures des isoflavones présents dans le raisin	32
Figure 12. Les modifications les plus courantes des stilbènes végétaux.....	33
Figure 13. Structures chimiques des acides hydroxycinnamiques	34
Figure 14. Structure des acides hydroxybenzoïques	35
Figure 15. Illustration simplifiée des réactions impliquées dans la formation et l'élimination des ERO.	43
Figure 16. Chronologie des principaux développements de la recherche sur les antioxydants alimentaires.....	47
Figure 17. Stimulation des macrophages RAW 264.7 par les LPS.....	52
Figure 18. Troubles liés à l'inflammation.....	53
Figure 19. Estimations de la prévalence mondiale du diabète.....	54
Figure 20. Physiopathologie du diabète de type 1	55
Figure 21. Physiopathologie du diabète de type 2.....	56
Figure 22. Critères de diagnostic du diabète	57
Figure 23. Réduction de la glycémie par l'inhibition de l'alpha glucosidase.....	59

Liste des tableaux

Tableau 1. Superficies des principaux pays viticoles	22
Tableau 2. Accumulation et concentrations en composés phénoliques au cours de la maturation	39
Tableau 3. Les principaux composés antioxydants dans les aliments	45

Liste des abréviations

ABTS : l'acide 2,2'-azino-bis (3-éthylbenzthiazoline-6-sulfonique)

ADN : acide désoxyribonucléique

AINS : anti-inflammatoires non stéroïdiens

ANR : anthocyanidine réductase

C : catéchine

CAT : catalase

CHI : chalcone isomérase

CHS : chalcone synthase

COX-2 : cyclooxygénase-2

DFR : dihydroflavonol réductase

DPm : degré de polymérisation moyen

DPPH : 1,1 -diphényl-2-picrylhydrazyle

EAG : équivalent acide gallique

EC : épicatechine

EC : équivalent catéchine

ECG : épicatechine gallate

EM3G : équivalent malvidine-3-O-glucoside

ERAs : espèces réactives de l'azote

ERO : espèces réactives de l'oxygène

F3'5'H : flavonoïde 3'5'-hydroxylase

F3'H : flavonoïde 3' –hydroxylase

F3H : flavanone 3-hydroxylase

FGT : flavonoïde 3-glucosyltransférase

FLS : flavonol synthase

FRAP : ferric reducing antioxidant power

GPx : glutathion peroxydase

GR : glutathion réductase

GSH : glutathion réduit

GSSG : glutathion disulfure

HbA1c : hémoglobine glyquée

IL-1 β : l'interleukine-1 β

IL-6 : l'interleukine-6

iNOS : Oxyde nitrique inductible synthase

LAR : leucoanthocyanidin réductase

LDOX : leucoanthocyanidine dioxygénase

LPS : lipopolysaccharides

MAO : monoamine oxydase

MS : matière sèche

NO : oxyde Nitrique

OMT : o-méthyltransférerase

PA : proanthocyanidines

PAL : phénylalanine ammoniac-lyase

PGE2 : prostaglandine E2

Prxs : les peroxyrédoxines

SOD : superoxyde dismutase

STS : stilbène synthase

TNF- α : facteur de nécrose tumorale α

UV : ultraviolet

Introduction générale

Vitis vinifera est l'espèce de vigne la plus cultivée dans le monde (Ky et al. 2014; Ky et Teissedre 2015; Yilmaz et al. 2015; Tkacz et al. 2019) avec une production mondiale de 78 millions tonnes de raisin en 2018 (<http://www.oiv.int/en/oiv-life/oiv-2019-report-on-the-world-vitivinicultural-situation>). Les baies de raisin sont utilisées comme fruits frais, produits secs ou pour la production de vin (Liang et al. 2011). En plus de leur importance économique, un nombre croissant d'avantages pour la santé ont été attribués aux raisins dus à la présence de composés phénoliques dans les différents tissus de la baie (Ali et al. 2011; Pandey et Rizvi 2009), principalement dans les pépins et les pellicules (Di Lecce et al. 2014; Ky et Teissedre 2015; Tkacz et al. 2019; Yilmaz et al. 2015).

Les polyphénols sont les métabolites secondaires les plus abondants dans le règne végétal (Ghani 2020; Lorrain et al. 2013). Ils constituent un groupe hétérogène de composés chimiques comprenant des cycles phénoliques portant un ou plusieurs groupes hydroxyles (Lorrain et al. 2013). Actuellement, il existe plus de 8000 polyphénols caractérisés et identifiés (Somerville, Bringans, et Braakhuis 2017). La composition et la concentration des composés phénoliques dépendent d'un certain nombre de facteurs tels que le stade de maturation (Bindon et al. 2013; Obreque-Slier et al. 2010), le climat, la variété et le type du sol (J. Kennedy 2002; Coklar 2017). Dans la plante, les polyphénols jouent un rôle important dans la croissance, la fertilité, la reproduction et dans les réactions de protection de la plante contre les stress biotiques et abiotiques (Gil-Muñoz et al. 2017). Ils possèdent aussi un large spectre d'activités biologiques (Plaza et al. 2018).

Les polyphénols sont connus pour leur rôle antioxydant (Balík et al. 2009; Rockenbach et al. 2011; Coklar 2017; Guendez 2005; Ky et Teissedre 2015; Lingua et al. 2016). Ils pourraient jouer un rôle important dans la prévention des maladies cardiovasculaires (Khurana et al. 2013; Amiot, Riollet, et Landrier 2009), neurodégénératives (Amiot, Riollet, et Landrier 2009; Richard et al. 2014), le diabète (Kadouh et al. 2016; Sales et al. 2012), l'inflammation (de plusieurs pathologies, telles que la polyarthrite rhumatoïde, l'inflammation des maladies intestinales et l'athérosclérose) (H. J. Lee, Kwon, et Kim 2018; Bak et al. 2013; Cheng et al. 2014; Du et al. 2018) et l'obésité (H. J. Lee, Kwon, et Kim 2018; Jack et al. 2019; Callcott et al. 2018). Enfin, leurs propriétés sont fortement liées à leurs structures chimiques (Plaza et al. 2018).

Les objectifs de ce travail sont de suivre l'évolution de la composition phénolique et des activités biologiques associées (antioxydante, anti-inflammatoire et anti-diabète) d'extraits de pépins et de pellicules au cours de la maturation de la baie de raisin.

Il est à noter que ce manuscrit est principalement rédigé sous forme d'articles.

Le **premier chapitre** expose la partie bibliographique concernant l'espèce *Vitis vinifera*, la biosynthèse et la structure des polyphénols de raisin, l'évolution de la composition phénolique (flavonols, stilbènes et anthocyanes) au cours de la maturation, ainsi leurs activités biologiques (activités antioxydante, anti-inflammatoire et anti-diabète).

Le **deuxième chapitre** est une revue bibliographique concernant les stilbènes du raisin et du vin et leurs effets comme agents de prévention de l'obésité.

Le **troisième chapitre** présente les travaux concernant l'activité antioxydante d'extraits phénoliques de pépins et de pellicules de trois cépages rouges (Merlot, Tannat et Syrah) au cours de la maturation. Des tests spectrophotométriques et électrochimiques ont été utilisés pour mesurer l'activité antioxydante. Le lien entre la composition polyphénolique et les activités mesurées a été étudié.

Le **quatrième chapitre** présente les résultats concernant l'activité anti-inflammatoire des extraits de pellicules de trois cépages rouges au cours de la maturation et le lien avec la composition phénolique.

Le **cinquième chapitre** traite l'inhibition de l'enzyme α -glucosidase par les extraits de pépins et de pellicules de Tannat au cours de la maturation en lien avec leurs compositions phénoliques.

La rédaction de ce manuscrit se terminera par une conclusion générale et des perspectives de travaux.

Chapitre 1 : Bibliographie

1. Le raisin au cours de la maturation

1.1. Le raisin

Le raisin est l'une des cultures fruitières les plus importantes au monde (Liang et al. 2012; Bozan, Tosun, et Özcan 2008) avec production d'environ 78 millions de tonnes en 2018 (Figure 1) sur 7.4 millions d'hectares de terres dédiées à sa culture (<http://www.oiv.int/fr/vie-de-loiv/bilan-de-loiv-2019-sur-la-situation-vitivinicole-mondiale>).

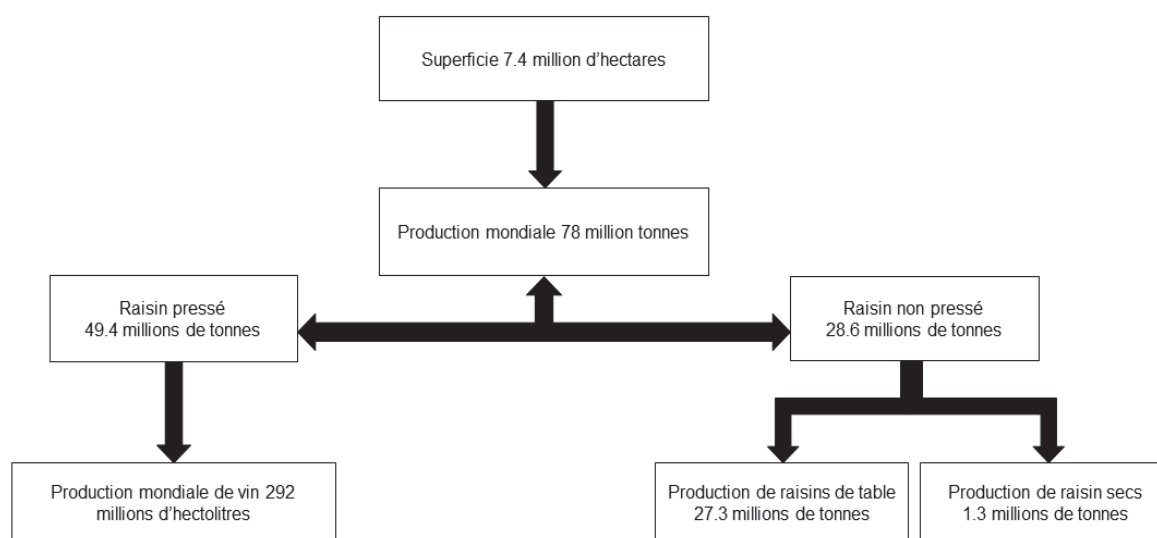


Figure 1. Production mondiale du raisin en 2018

Le raisin est produit par les plantes du genre *Vitis* qui regroupe environ 60 espèces de vignes. *Vitis vinifera* est l'espèce la plus cultivée et représente plus de 90% des baies de raisin sur le marché (Venkitasamy et al. 2019). Les baies sont consommées sous forme de fruits frais ou d'autres produits transformés, notamment le jus de raisin, la confiture, le vin, les raisins secs le vinaigre et l'huile de pépins de raisin (Venkitasamy et al. 2019). Les baies de raisin contiennent trois principaux types de tissus : la pellicule, les pépins et la pulpe (Figure 2) (Adams 2006; J. Kennedy 2002).

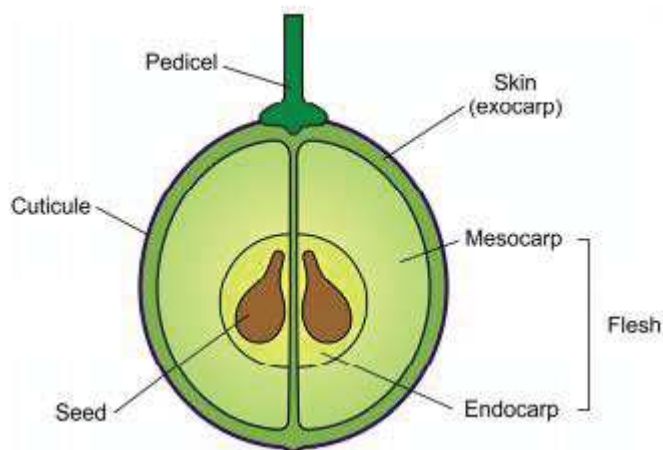


Figure 2. Structure d'une baie du raisin (Conde et al., s. d.)

Environ 10 000 cépages sont disponibles dans le monde, 13 variétés sont cultivées sur plus d'un tiers de la superficie viticole mondiale et 33 variétés sur environ 50%. Certaines variétés cultivées dans plusieurs pays sont appelées variétés internationales. Par exemple, le Cabernet-Sauvignon est l'un des cépages les plus cultivés au monde, couvrant plus de 5% de la superficie viticole mondiale (Venkitasamy et al. 2019). En 2019, l'Espagne est classée comme le premier producteur de raisins dans le monde, avec 13.1% de la production totale mondiale suivi par la Chine, la France et l'Italie (Tableau1) (<http://www.oiv.int/public/medias/7302/fr-note-de-conjoncture-2019-du-secteur-vitivinicole-mondial>).

Tableau 1. Superficies des principaux pays viticoles (millions d'hectares)

Pays/Année	2015	2016	2017	2018	2019	2019 % monde
Espagne	974	975	968	972	966	13.1
Chine	859	807	830	855	855	11.5
France	785	786	788	792	794	10.7
Italia	685	693	699	701	708	9.6
Turquie	497	468	448	448	436	5.9

Etats-Unis	446	439	434	408	408	5.5
Argentine	225	224	222	218	215	2.9
Chili	214	209	207	203	200	2.7
Portugal	204	195	194	192	195	2.6
Roumanie	191	191	191	191	191	2.6
Iran	217	168	153	177	177	2.4
Inde	129	131	147	149	149	2
Australie	147	145	145	146	146	2
Afrique de Sud	133	130	128	123	122	1.7
Grèce	107	105	106	106	106	1.4
Allemagne	103	102	103	103	103	1.4
Russie	85	89	90	93	95	1.3

1.2. Le développement de la baie du raisin

L'évolution dans le temps et la croissance des raisins *Vitis Vinifera* est bien décrit dans la littérature (J. Kennedy 2002; Adams 2006; Conde et al., s. d.). Les baies de raisin subissent une complexe série de changements physiques et chimiques au cours de leur développement qui peut être divisé en deux phases de croissance sigmoïdales séparées par une phase de latence (Figure 3) (Fortes et Pais 2016; J. Kennedy 2002; Deluc et al. 2007; B. G. Coombe 1992; Coombe et McCARTHY 2000; Dokoozlian et Kliewer 1996).

La première phase : elle consiste à la formation des embryons de pépins et de péricarpe. Cette étape est caractérisée par une croissance exponentielle rapide de la baie qui se dilate en volume du fait de la multiplication cellulaire. La biosynthèse des tanins et des acides hydroxycinnamiques et l'accumulation des acides organiques comme le tartrate et le malate débutent à cette période (Fortes et Pais 2016; Conde et al., s. d.; Deluc et al. 2007).

La deuxième phase : c'est une phase de transition au cours de laquelle il n'y a pas d'augmentation de la taille des baies. La véraison marque le début de la troisième phase par l'initiation du développement de la couleur dû à l'accumulation des anthocyanes dans le raisin rouge (Fortes et Pais 2016; Deluc et al. 2007).

La troisième phase : la maturation, implique une croissance supplémentaire des baies en raison de l'expansion cellulaire. Il y a également une diminution de la teneur en malate et une accumulation des sucres (principalement glucose et fructose) ainsi que des anthocyanes (Fortes et Pais 2016). Les baies passent d'un état où elles sont petites, dures et acides à un état où elles sont plus grosses, plus sucrées, moins acides et fortement colorées et aromatiques (Conde et al., s. d.).

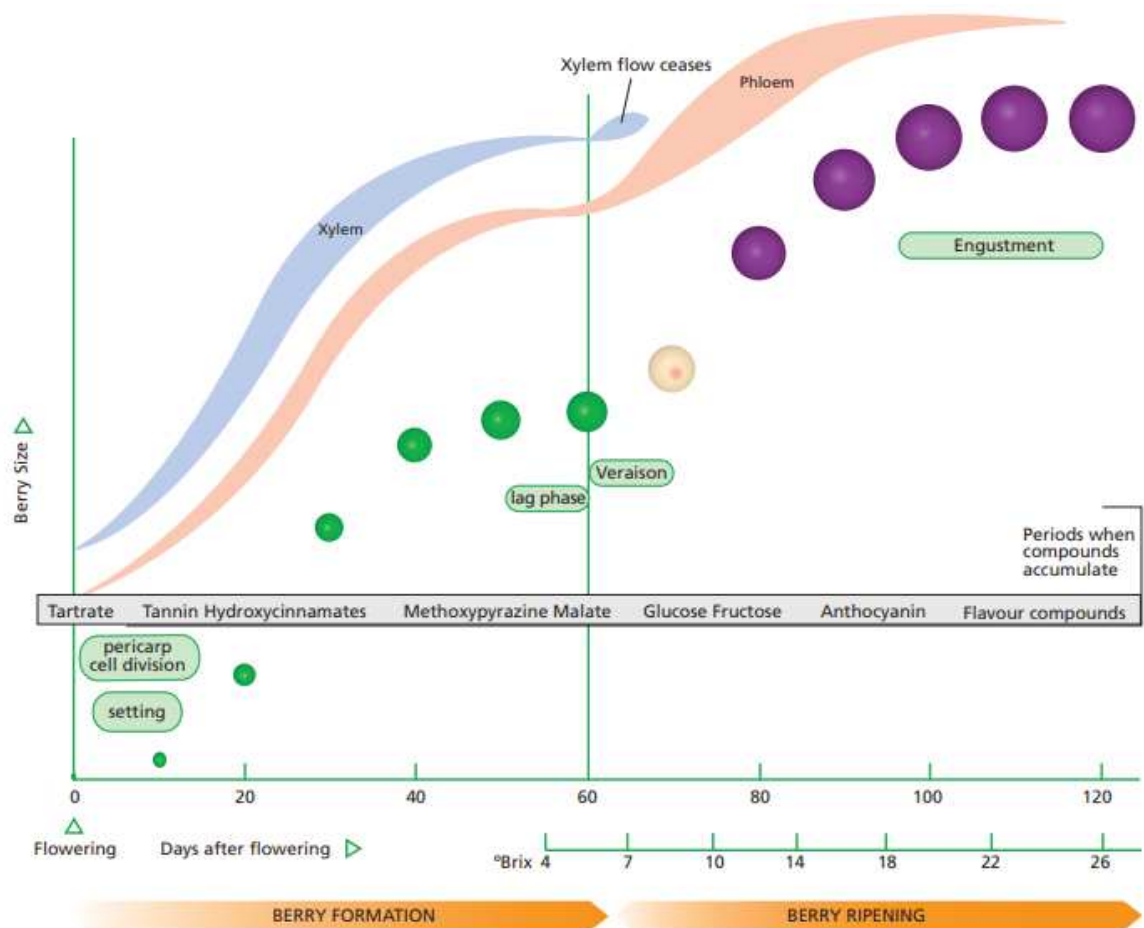


Figure 3. Diagramme montrant la taille et la couleur relative des baies au cours de la maturation (J. Kennedy 2002)

2. Les polyphénols du raisin

Les polyphénols sont les métabolites secondaires les plus abondants du règne végétal (Ghani 2020; Lorrain et al. 2013; Kundu, Talukder, et Sen Raychaudhuri 2019). Ils sont présents en plus grande quantité dans les raisins après les glucides et les acides (Nawaz et al. 2006) et sont localisés principalement dans les pépins et les pellicules (Pantelić et al. 2016; Fanzone et al. 2011; Giuffrè 2013; J. A. Kennedy et al. 2001; Obreque-Slier et al. 2013). Les polyphénols ont une structure commune possédant un noyau aromatique avec un ou plusieurs groupements hydroxyles (Quideau et al. 2011; Kardum et Glibetic 2018; Ghani 2020). Il existe environ 8000 composés (Somerville, Bringans, et Braakhuis 2017; J. Santos et al. 2014; Chandrasekara et Shahidi 2018) solubles dans l'eau et dans les solvants organiques (Ashraf et al. 2018). On distingue deux grandes familles: les flavonoïdes et les non flavonoïdes (Tkacz et al. 2019; Obreque-Slier et al. 2013).

Les polyphénols sont synthétisés à travers la voie des phénylpropanoïdes (Adams 2006), en réponse à des stimuli externes (Cavaliere et al. 2008; Versari et al. 2001) et ils ont diverses activités (Bak et al. 2013; Cheng et al. 2014). Ils protègent les plantes contre les facteurs de stress biotiques et abiotiques (Błaszczuk, Sady, et Sielicka 2019; Gil-Muñoz et al. 2017). La plupart de ces métabolites sont responsables des propriétés organoleptiques et qualitatives des aliments provenant de ces plantes (del Llaudy et al. 2008; Asproudi et al. 2015; Rodríguez Montealegre et al. 2006). Ces composés sont connus pour leurs impacts bénéfiques sur la santé et par leurs nombreuses activités biologiques cardioprotective (Khurana et al. 2013; Amiot, Riollet, et Landrier 2009), anti-inflammatoire (H. J. Lee, Kwon, et Kim 2018; Bak et al. 2013; Cheng et al. 2014; Du et al. 2018; Terra et al. 2007), anti-diabète (Kadouh et al. 2016; Sales et al. 2012) et principalement antioxydante (Balík et al. 2009; Aguirre et al. 2010; Rockenbach et al. 2011; Bozan, Tosun, et Özcan 2008).

2.1. Biosynthèse générale, familles de composés (flavonoïdes/non-flavonoïdes)

La biosynthèse des phénylpropanoïdes commence habituellement par l'acide aminée phénylalanine issue de la voie du shikimate (Conde et al., s. d.; Kundu, Talukder, et Sen Raychaudhuri 2019). Cette voie est responsable de la production de la phénylalanine et d'autres acides aminés aromatiques comme la tyrosine, le tryptophane (Conde et al., s. d.), l'acide gallique et l'acide cinnamique (Fatland et al. 2002). La première enzyme responsable de la synthèse phénolique est la PAL (phényl ammoniac lysase) qui convertit la phénylalanine en acide *trans*-cinnamique (Conde et al., s. d.; Kundu, Talukder, et Sen Raychaudhuri 2019). Ce composé subit une série de transformations entraînant la formation de précurseurs de plusieurs composés phénoliques simples, comme les acides phénols. L'incorporation de malonyl-CoA avec le coumaroyl-CoA donne la naringénine chalcone par l'enzyme chalcone synthase (CHS). La chalcone est isomérisée par la chalcone flavanone isomérase (CHI) en une flavanone ; l'intermédiaire de base pour les autres classes et sous-classes de flavonoïdes par l'intervention des différentes enzymes (Figure 4) (Bohm 1998; Tsao, Khanizadeh, et Dale 2006; Tsao 2010). Dans le cas des stilbènes c'est l'enzyme stilbène synthase (STS) qui intervient (Chong, Poutaraud, et Hugueney 2009).

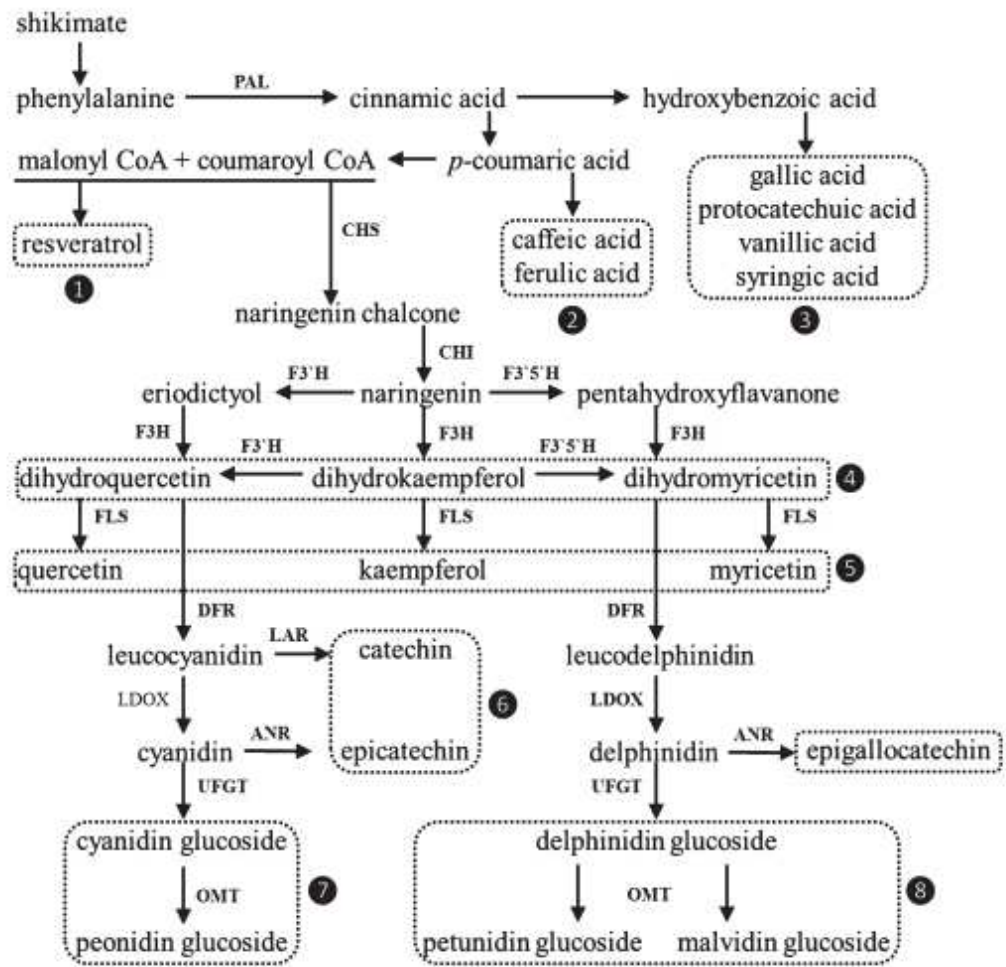


Figure 4. La voie biosynthétique des principaux composés phénoliques du raisin. Sous-groupes: 1, stilbènes; 2, acides hydroxycinnamiques; 3, acides hydroxybenzoïques; 4, dihydroflavonols; 5, flavonols; 6, flavanols; 7, anthocyanes dihydroxylés; 8, anthocyanes trihydroxylées. Abréviations: ANR, anthocyanidine réductase; CHI, chalcone isomérise; CHS, chalcone synthase; DFR, dihydroflavonol réductase; F3H, flavanone 3-hydroxylase; F3' H, flavonoïde 3' -hydroxylase; F3'5' H, flavonoïde 3'5'-hydroxylase; FLS, flavonol synthase; LAR, leucoanthocyanidin réductase; LDOX, leucoanthocyanidine dioxygénase; OMT, o-méthyltransférase; PAL, phénylalanine ammoniac-lyase; UFGT, flavonoïde 3-glucosyltransférase (Berli et al. 2011).

2.1.1. Les flavonoïdes

Les flavonoïdes sont les composés phénoliques les plus abondants chez les végétaux (Conde et al., s. d.; Stoclet et Schini-Kerth 2011), ils présentent un large éventail de fonctions importantes telles que la pigmentation, les interactions plante-pathogène, la fertilité et la protection contre les ultraviolets (UV). Ils sont très souvent colorés et principalement présents sous forme glycosylée (Richard et al. 2014; Viñas et Campillo 2019). Leur structure de base se compose de deux cycles aromatiques avec six atomes de carbone (cycles A et B) lié par un hétérocycle comprenant trois atomes de carbone (cycle C) (Stoclet et Schini-Kerth 2011; Viñas et Campillo 2019). Ce groupe est subdivisé en plusieurs familles en fonction de l'état d'oxydation du cycle C (Ghedira 2005) : on trouve les flavanols, les anthocyanes, les flavanones, les flavonols, les flavones et les isoflavones (Figure 5) (Stoclet et Schini-Kerth 2011).

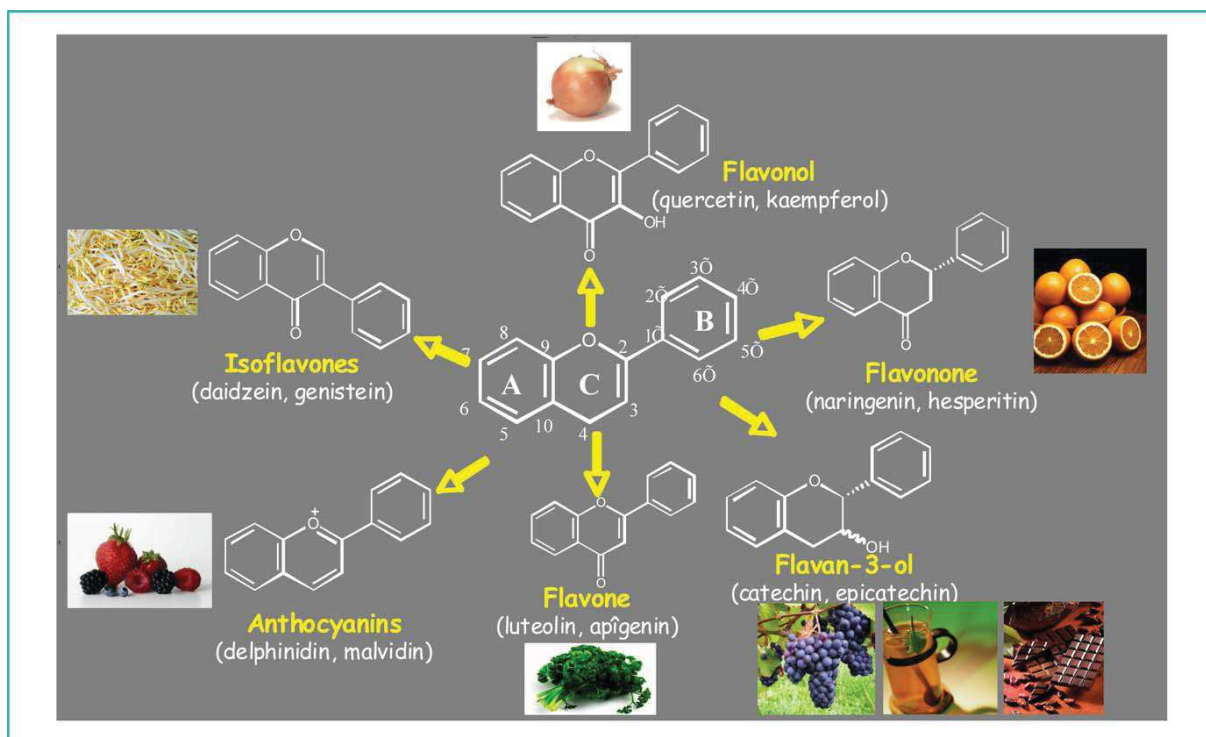


Figure 5. Structure des principaux flavonoïdes (Stoclet et Schini-Kerth 2011)

2.1.1.1. Les flavanols

C'est un groupe important de composés des flavonoïdes, ils se trouvent à la fois dans les pépins et les pellicules, mais les pépins en contiennent beaucoup plus que

les pellicules et leurs compositions sont différentes aussi (González-Manzano, Rivas-Gonzalo, et Santos-Buelga 2004; Pantelić et al. 2016; Bozan, Tosun, et Özcan 2008; del Llaudy et al. 2008). Ils sont responsables de la sensation d'astringence (sensation tactile de sécheresse de la muqueuse buccale) en interagissant avec les protéines salivaires (Gonçalves et al., 2011). Ils comprennent non seulement des monomères mais également des oligomères et des polymères (tanins condensés ou des proanthocyanidines (PA)). Ces derniers sont divisés en procyanidines (dérivées de la catéchine, de l'épicatéchine et de leurs esters galliques) ou prodelfphinidines (dérivées de gallocatéchine, épigallocatéchine et leurs dérivés galloylés) (Viñas et Campillo 2019). Les flavanols présentent différents degrés d'hydroxylation sur les noyaux A, B et la position 3 sur le C et peuvent être estérifiés avec de l'acide gallique (Mateus et al. 2001). Les teneurs en flavan-3-ols galloylés sont également plus importants dans les pépins que dans les pellicules (Obreque-Slier et al. 2010). Les monomères de flavanols les plus courants sont la catéchine, l'épicatéchine, la catéchine gallate, l'épigallocatéchine gallate, l'épicatéchine gallate et l'épigallocatéchine (Figure 6) (Spranger et al. 2008; Viñas et Campillo 2019). Le degré de polymérisation est relié à la longueur de la chaîne de proanthocyanidine et est plus élevé au niveau des pellicules par rapport aux pépins (Lorrain et al. 2013).

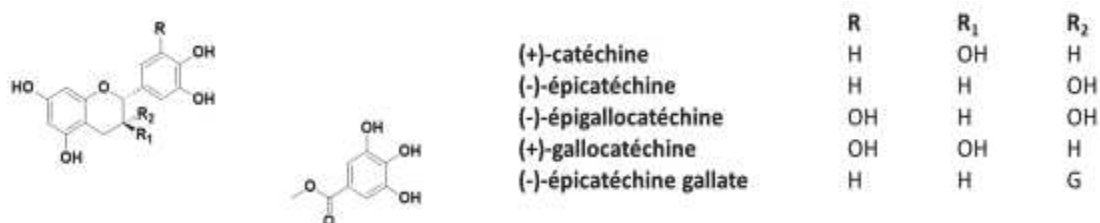


Figure 6. Les monomères de flavanols présents dans le raisin

2.1.1.2. Les anthocyanes

Ces pigments naturels appartiennent à la famille des flavonoïdes (de Pascual-Teresa et Sanchez-Ballesta 2008) et sont responsables de la couleur des plantes (Viñas et Campillo 2019). Dans le raisin, elles se trouvent uniquement dans les pellicules (del Llaudy et al. 2008). Les anthocyanes sont présentes principalement sous forme d'hétérosides : la forme aglycone des anthocyanes, également appelée

anthocyanidine, est structurellement basée sur l'ion flavilium ou 2-phénylbenzopyrilium avec des groupements hydroxyles et méthoxyles dans différentes positions. Selon le nombre et la position des substituants hydroxyles et méthoxyles, différentes anthocyanidines ont été décrites dans la littérature (Lorrain et al. 2013; de Pascual-Teresa et Sanchez-Ballesta 2008). Parmi celles-ci, on cite : la delphinidine, la cyanidine, la pétunidine, la pélargonidine, la peonidine et la malvidine (Figure 7). Cependant, les glycosides de malvidine sont les anthocyanes les plus caractéristiques du raisin rouge et des produits dérivés (Mulero, Pardo, et Zafrilla 2010).

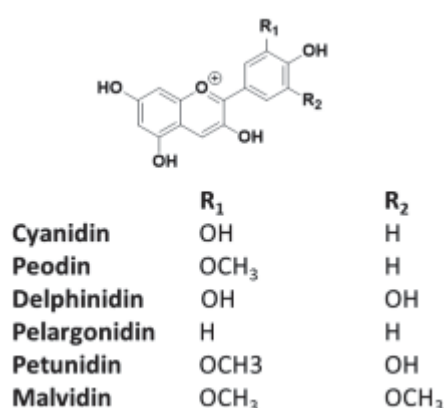


Figure 7. Structures des anthocyanes les plus présentes dans le raisin

2.1.1.3. Les flavanones

Ils contiennent un atome d'oxygène en position C4, en plus d'avoir le noyau C saturé. Ces polyphénols se trouvent principalement lié à 1 ou 2 sucres, et moins fréquemment sous forme d'aglycone (Figure 8). On les trouve majoritairement dans les pellicules d'agrumes aussi bien dans le jus (Calderón-Oliver et Ponce-Alquicira, s. d.).

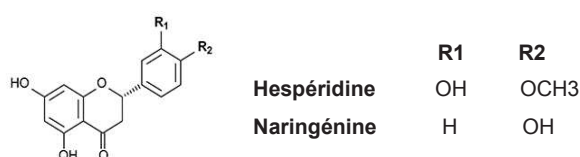


Figure 8. Structures des flavanones présents dans le raisin

2.1.1.4. Les flavonols

Les flavonols ont une double liaison entre les carbones C2 et C3, en plus d'un atome d'oxygène en position C4 et un hydroxyle en C3. Ils sont considérés parmi les phénols les plus présents dans les pellicules de raisin (Pantelić et al. 2016) mais ils sont présents en quantités beaucoup plus faible que les anthocyanes (Mulero, Pardo et Zafrilla 2010). Dans les fruits, les flavonols augmentent la couleur en stabilisant la forme colorée de la molécule d'anthocyanine (Singh Brar, Singh et Swinny 2008). Les flavonols sont généralement liés aux sucres comme le glucose, le rhamnose, le galactose et l'arabinose (Calderón-Oliver et Ponce-Alquicira, s. d.). Les principaux composés représentatifs sont la quercétine et le kaempférol (Figure 9) (Viñas et Campillo 2019).

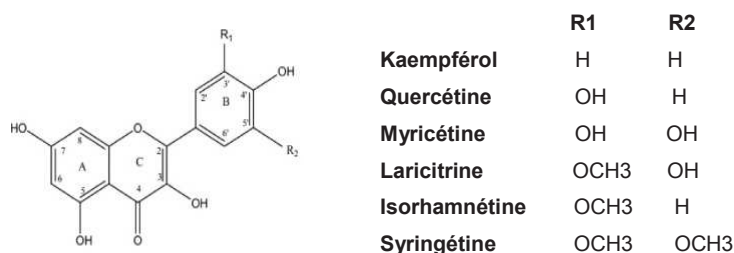


Figure 9. Structures des flavonols présents dans le raisin

2.1.1.5. Les flavones

Les flavones, nommés aussi 2-phénylchromones (Kshatriya, Jejurkar, et Saha 2018), possèdent des structures cycliques similaires aux autres flavonoïdes avec une double liaison entre les carbones C2 et C3 et sans groupement hydroxyle en C3 (Chandrasekara et Shahidi 2018; Hostetler, Ralston, et Schwartz 2017). Les flavones les plus connues sont : l'apigénine, la lutéoline, et la chrysin (Figure 10) (Pietta 2000).

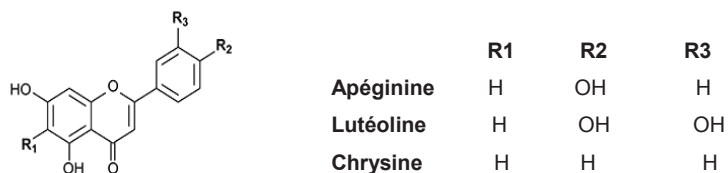


Figure 10. Structures des flavones présents dans le raisin

2.1.1.6. Les isoflavones

Les isoflavones sont des isomères de flavones ; seulement le groupe phényle (noyau B) est lié en position 3 du noyau C au lieu de la position 2 pour les flavones (Figure 11) (Calderón-Oliver et Ponce-Alquicira, s. d.).

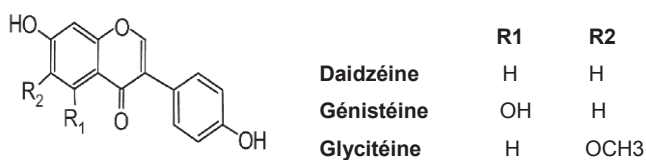


Figure 11. Structures des isoflavones présents dans le raisin

2.1.2. Les non- flavonoïdes

La famille des non flavonoïdes comprend les stilbènes et les acides phénols (hydroxycinnamiques et hydroxybenzoïques) (Adams 2006).

2.1.2.1. Les stilbènes

Les stilbènes sont des phytoalexines de structure chimique C6-C2-C6 (Richard et al. 2014b; Kiselev et al. 2019) situés essentiellement dans la pellicule de raisin et ils se trouvent principalement sous forme glycosylés (Geana et al. 2015). Ils sont synthétisés par les plantes en tant que composés de défense contre les stress biotiques et abiotiques (Cacho et al. 2013). Les stilbènes peuvent être sous forme de monomères, dimères ou oligomères (Chong, Poutaraud, et Hugueney 2009). Le

resvératrol est le stilbène le plus connu dans le monde (Careri et al. 2003). Il subit plusieurs modifications (glycosylation, methoxylation, oligomérisation et isoprénylation) (Figure 12) pour donner des composés plus complexes (Chong, Poutaraud, et Huguency 2009). Les stilbènes monomères les plus abondants dans les baies sont le *trans*-resvératrol et le *trans*-picéide (Błaszczuk, Sady et Sielicka 2019).

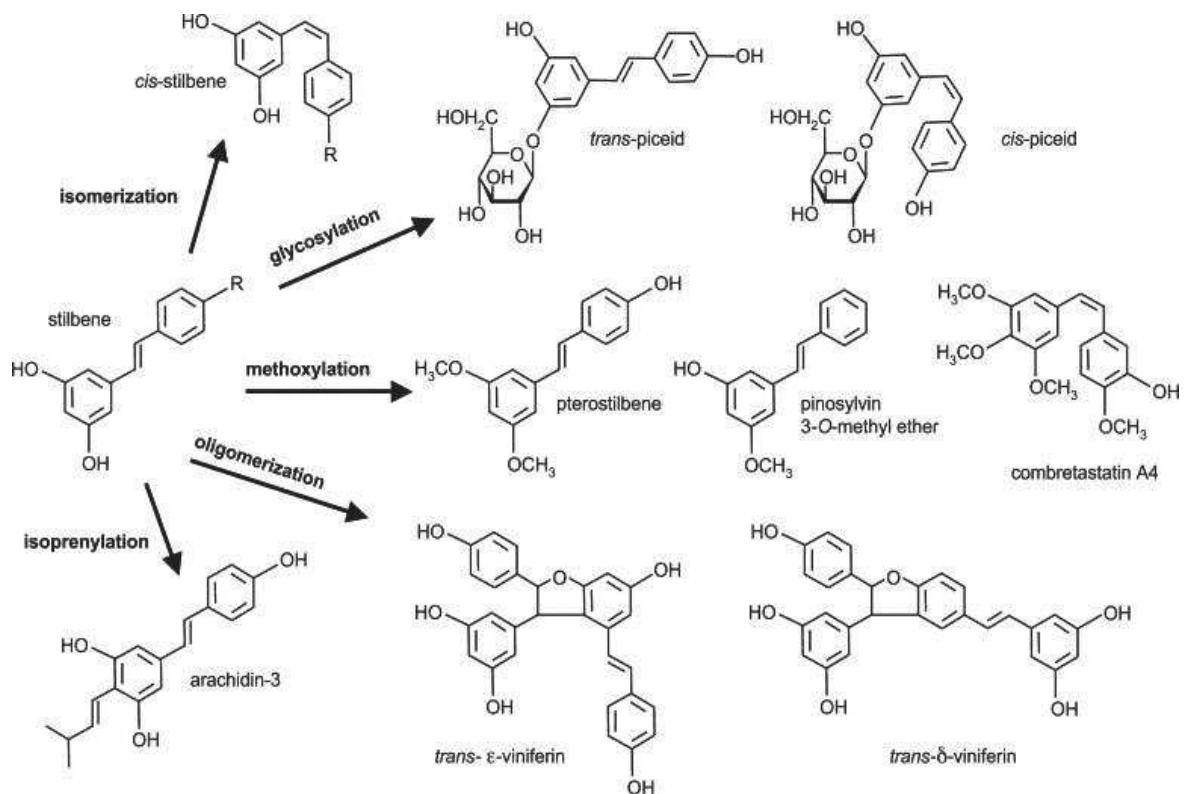


Figure 12. Les modifications les plus courantes des stilbènes végétaux (Chong, Poutaraud, et Huguency 2009)

2.1.2.2. Les acides phénols

C'est une classe diversifiée de composés phénoliques divisée en acides hydroxybenzoïques et acides hydroxycinnamiques (Chandrasekara et Shahidi 2018; Dzah et al. 2020; Fuentes et Palomo 2014; Ghani 2020; Santos et al. 2014; Kumar et Goel 2019; Rashmi et Negi 2020) en fonction du nombre et de la position des groupements hydroxyles sur le cycle aromatique (Rashmi et Negi 2020).

❖ Les acides hydroxycinnamiques

Les acides hydroxycinnamiques sont présents principalement dans les céréales, les fruits et les légumes (Alam et al. 2016; Fuentes et Palomo 2014). Ils ont un squelette carboné C6-C3 avec une double liaison dans la chaîne latérale (El-Seedi et al. 2012; Rashmi et Negi 2020). Les principaux acides hydroxycinnamiques sont les acides caféique, férulique, sinapique, *p*-coumarique (Figure 13) (Chandrasekara et Shahidi 2018; El-Seedi et al. 2012), cinnamique, *o*-coumarique et *m*-coumarique (El-Seedi et al. 2012).

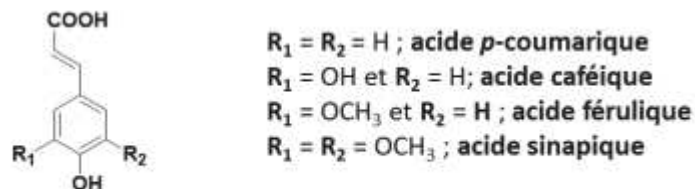


Figure 13. Structures chimiques des acides hydroxycinnamiques

❖ Les acides hydroxybenzoïques

Les acides hydroxybenzoïques sont des dérivés de l'acide benzoïque avec un squelette carboné de type C6-C1 (Rashmi et Negi 2020). Les acides hydroxybenzoïques les plus présents sont l'acide vanillique, syringique, gallique et protocatéchique (Figure 14) (Rashmi et Negi 2020; Chandrasekara et Shahidi 2018).

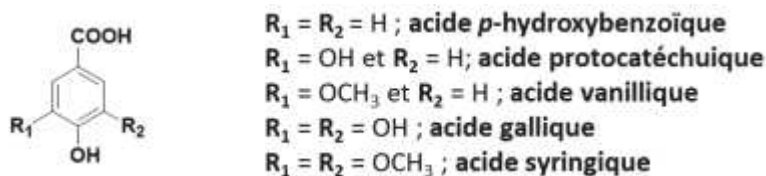


Figure 14. Structure des acides hydroxybenzoïques

2.2. Accumulation et concentration en composés phénoliques au cours de la maturation du raisin rouge

Le développement des baies du raisin au cours du temps est un processus dynamique qui implique une série complexe de changements génétiques, moléculaires et chimiques (Deluc et al. 2007). L'évolution de la concentration des composés phénoliques de raisin est fortement liée au stade de maturité (Tableau 2).

2.2.1. Les polyphénols totaux

La teneur en polyphénols dépend des facteurs climatiques et géographiques, des pratiques culturales, des stades de maturité et de la variété (Lorrain, Chira, et Teissedre 2011; Coklar 2017; Ky et al. 2014; Niu et al. 2017; Yilmaz et al. 2015; Pantelić et al. 2016). La teneur en polyphénols totaux est beaucoup plus élevée pour les pépins que pour les pellicules (Coklar 2017; C. Xu et al. 2010; Rodríguez Montealegre et al. 2006). Elle diminue après la véraison dans les deux tissus (pépins et pellicules) (Obreque-Slier et al. 2010).

2.2.2. Les flavanols

Les flavanols sont les polyphénols les plus abondants dans les baies du raisin (Adams 2006). Ils sont présents aussi bien dans les pépins que dans les pellicules (del Llaudy et al. 2008).

❖ **Les pépins** : ils contiennent la catéchine (C), l'épicatéchine (EC) et l'épicatéchine gallate (ECG) ainsi que les procyanidines qui sont des polymères de flavanols (Ristic et Iland 2005; González-Manzano, Rivas-Gonzalo, et Santos-Buelga 2004; del Llaudy et al. 2008; Rolle et al. 2011; Cadot, Miñana Castelló, et Chevalier 2006). L'accumulation des flavanols commence au début du développement des pépins, leur concentration est maximale à la véraison puis elle diminue à la maturité (Downey, Harvey, et Robinson 2003; Ristic et Iland 2005; J. A. Kennedy, Matthews, et Waterhouse 2000b; Rolle et al. 2011). La concentration des flavanols dépend de la variété, des variétés comme le pinot noir, le grenache, et le tempranillo ont des teneurs élevées en proanthocyanidines tandis que d'autres comme la syrah, et le merlot ont des teneurs faibles (Ristic et Iland 2005).

❖ **Les pellicules** : elles contiennent l'épigallocatechine et une proportion beaucoup plus faible de l'épicatéchine-3-gallate plus les proanthocyanidines (González-Manzano, Rivas-Gonzalo, et Santos-Buelga 2004; del Llaudy et al. 2008; Rolle et al. 2011). La teneur en flavanols est plus élevée avant la véraison qu'après (Asproudi et al. 2015; Lorrain, Chira, et Teissedre 2011; Obreque-Slier et al. 2010), elle diminue lentement de la véraison à la maturité (Adams 2006). La diminution de la teneur après la véraison est due à la déviation des métabolites intermédiaires (cyanidines et delphinidines) vers la synthèse des anthocyanes ou à l'oxydation de ces composés (Asproudi et al. 2015). Les proanthocyanidines présentent un degré de polymérisation plus élevé pour les pellicules que pour les pépins (del Llaudy et al. 2008; Adams 2006).

2.2.3. Les anthocyanes

Les anthocyanes sont localisés uniquement dans les pellicules des cépages rouges. La synthèse des anthocyanes commence à partir de la véraison, qui est considérée comme le début de la maturation des baies (Adams 2006; Shahab et al. 2020). La malvidine -3-O- glucoside est la principale anthocyane présente dans les raisins (Adams 2006; Lorrain, Chira, et Teissedre 2011; Fournand et al. 2006), elle représente 44% et 55% des anthocyanes totales pour le Merlot et le Cabernet Sauvignon, respectivement (Rodríguez Montealegre et al. 2006) suivie de la

delphinidine -3-O-glucoside. Les anthocyanes s'accumulent dans les pellicules des baies de la véraison à la maturité du raisin (Fournand et al. 2006; Giuffrè 2013; J. A. Kennedy, Matthews, et Waterhouse 2002; Esteban, Villanueva, et Lissarrague 2001; Delgado et al. 2004). D'autres études montrent que la concentration des anthocyanes augmente rapidement de la véraison au stade intermédiaire (entre la véraison et la maturité) et plus lentement par la suite (Gil-Muñoz et al. 2011; Lorrain, Chira, et Teissedre 2011). Il y a ensuite une diminution juste avant la récolte et/ou pendant la surmaturation (Ryan et Revilla 2003; Ristic et Iland 2005; Mateus et al. 2001; Singh Brar, Singh, et Swinny 2008; Gil-Muñoz et al. 2011; Fournand et al. 2006; Rolle et al. 2011). L'accumulation des anthocyanes est liée à différents facteurs tels que la variété (Ryan et Revilla 2003; Gil-Muñoz et al. 2011), le climat, l'irrigation et le sol (Ryan et Revilla 2003; del Llaudy et al. 2008; Esteban, Villanueva, et Lissarrague 2001; Pérez-Magariño et González-San José 2004).

2.2.4. Les stilbènes

Les stilbènes se trouvent principalement dans les pellicules du raisin (Gatto et al. 2008; Jeandet et al. 2002; Babazadeh et al. 2017). Ils sont considérés comme des phytoalexines car ils sont associés à la résistance des plantes aux maladies et leur synthèse est souvent due à une réponse aux attaques par des agents phytopathogènes et d'autres facteurs de stress comme l'irradiation UV (Błaszczyk, Sady, et Sielicka 2019; Jeandet et al. 2002; Gatto et al. 2008). Le stilbène le plus important dans les raisins est le resvératrol et sa forme glycosylée, le picéide. La concentration en stilbènes augmente au cours de la maturation (Gatto et al. 2008; Geana et al. 2015).

2.2.5. Les flavonols

Les flavonols sont moins abondants que les flavanols et les anthocyanes. Ils se présentent majoritairement dans le raisin sous forme glycosylés. Leur concentration dépend de l'exposition à la lumière, les baies exposées au soleil ayant des niveaux

plus élevés que les baies non exposées (Adams 2006). La teneur en flavonols augmente au cours de la maturation (Giuffrè 2013).

Tableau 2. Accumulation et concentrations en composés phénoliques au cours de la maturation

Pépins	Avant véraison	Autour de la véraison	Après véraison	Références
Polyphénols totaux				
Cabernet Sauvignon	/	56.1 ± 1.8 mg EAG /g MS	39.1 ± 2.5 mg EAG /g MS	(Lorrain, Chira, et Teissedre 2011)
Merlot	/	79.5 ± 0.6 mg EAG /g MS	45.3 ± 0.5 mg EAG /g MS	(Lorrain, Chira, et Teissedre 2011)
Malbec	/	38.3 ± 0.7 mg EAG /g	26.7 ± 2.5 mg EAG /g	(Fanzone et al. 2011)
Flavanols				
Cabernet Sauvignon	/	94.0 ± 4.8 mg/g MS	90.1 ± 4.0 mg/g MS	(Lorrain, Chira, et Teissedre 2011)
Merlot	/	138.9 ± 5.3 mg/g MS	92.2 ± 4.5 mg/g MS	(Lorrain, Chira, et Teissedre 2011)
Malbec	/	123 ± 14.2 mg EC/g	125.2 ± 3.6 mg EC/g	(Fanzone et al. 2011)
Tannat	/	1576 ± 81 mg/kg	1931 ± 216 mg/kg	(Boido et al. 2011)
DPM				
Cabernet Sauvignon	/	20.5	16.1	(Lorrain, Chira, et Teissedre 2011)
Merlot	/	15 .1	11.5	(Lorrain, Chira, et Teissedre 2011)
Syrah	9.2	6	5.7	(J. A. Kennedy et al. 2000)

Pellicules	Avant véraison	Autour de la véraison	Après véraison	Références
Polyphénols totaux				
Cabernet Sauvignon	/	31.6 ± 0.72 mg EAG/g MS	29.5 ± 0.0 mg EAG /g MS	(Lorrain, Chira, et Teissedre 2011)
Merlot	/	30 ± 0.4 mg EAG /g MS	31.8 ± 0.2 mg EAG /g MS	(Lorrain, Chira, et Teissedre 2011)
Malbec	/	6 ± 0.1 mg EAG /g	10.5 ± 1.5 mg EAG /g	(Fanzone et al. 2011)
Flavanols				
Cabernet Sauvignon	/	108.2 ± 9.5 mg/g MS	57.4 ± 0.4 mg/g MS	(Lorrain, Chira, et Teissedre 2011)
Merlot	/	95.9 ± 1.7 mg/g MS	63.8 ± 0.1 mg/g MS	(Lorrain, Chira, et Teissedre 2011)
Malbec	/	20.9 ± 2.7 mg EC/g	7.7 ± 0.7 mg EC/g	(Fanzone et al. 2011)
Tannat	/	30 ± 4 mg/kg	38 ± 11 mg/kg	(Boido et al. 2011)
Flavonols				
Malbec	/	148 ± 16.3 mg/Kg de pellicules	288.6 ± 22.3 mg/Kg de pellicules	(Fanzone et al. 2011)
Nerello		11.44 mg/kg	35.79 mg/kg	(Giuffrè 2013)
Stilbènes				
Pinot noir	/	2.9 ± 0.1 mg/kg	8.5 ± 0.3 mg/kg	(Geana et al. 2015)
Merlot	/	1.6 ± 0.1 mg/kg	4.4 ± 0.2 mg/kg	(Geana et al. 2015)
Cabernet Sauvignon		0.15 ± 0.04 mg/kg	0.67 ± 0.06 mg/kg	(Geana et al. 2015)
Malbec	/	1.1 ± 0.1 mg/Kg de pellicules	7.2 ± 0.7 mg/Kg de pellicules	(Fanzone et al. 2011)
Anthocyanes				

Cabernet Sauvignon	/	4.7 ± 0.7 mg/g MS	19.8 ± 1.9 mg/g MS	(Lorrain, Chira, et Teissedre 2011)
Merlot	/	7.4 ± 0.7 mg/g MS	22.2 ± 0.5 mg/g MS	(Lorrain, Chira, et Teissedre 2011)
Malbec	/	1.3 ± 0.1 mg EM3G/g	4.3 ± 0.6 mg EM3G/g	(Fanzone et al. 2011)
Tannat		1695 ± 14 mg/kg	2528 ± 183 mg/kg	(Boido et al. 2011)
Cabernet Sauvignon	/	411 ± 22 mg/L	728 ± 30 mg/L	(Bindon et al. 2013)
DPm				
Cabernet Sauvignon	/	7.15 ± 0.1	8.39 ± 0.07 ^d	(Bindon et al. 2013)
Cabernet Sauvignon		48.3	11.3	(Lorrain, Chira, et Teissedre 2011)
Merlot		49.1	37.8	(Lorrain, Chira, et Teissedre 2011)
Syrah	7.3 ± 0.1	11.3 ± 0.1	27 ± 0.1	(J. A. Kennedy et al. 2001)
Cabernet Sauvignon	/	15	30	(J. A. Kennedy, Matthews, et Waterhouse 2002)

3. Les activités biologiques de raisin

3.1. L'activité antioxydante

3.1.1. Espèces réactives oxygénées et stress oxydant

❖ Les espèces réactives

Il existe deux grands types de molécules réactives au niveau cellulaire: les espèces réactives vis-à-vis de l'oxygène (ERO) et les espèces réactives vis-à-vis de l'azote (ERA) (Mirończuk-Chodakowska, Witkowska, et Zujko 2018; Rizzo et al. 2010). Les deux types comprennent les radicaux libres qui sont des espèces chimiques réactives ayant un électron non apparié ou plus dans leurs orbitales ultrapériphériques (Johansen et al. 2005; Losada-Barreiro et Bravo-Díaz 2017). Cette configuration est instable ce qui rend les radicaux libres réactifs (Johansen et al. 2005; Marfella et al. 2014). La principale source endogène de la production des EROs est la chaîne respiratoire mitochondriale (Figure 15) (Losada-Barreiro et Bravo-Díaz 2017; Dudonné et al. 2009) qui utilise environ 80 % ou 90 % de l'oxygène consommé (Losada-Barreiro et Bravo-Díaz 2017). De plus, les espèces réactives pourraient résulter de sources exogènes comme l'exposition au rayonnement ionisant et aux polluants (Chevion, Roberts, et Chevion, s. d.).

Les EROs interviennent dans la signalisation intercellulaire qui régule diverses fonctions physiologiques (Tai et Ascoli 2011; Chedea et Pop 2019; Mirończuk-Chodakowska, Witkowska, et Zujko 2018; Valko et al. 2007), elles jouent un rôle important dans la protection des cellules contre les infections en réduisant les agents pathogènes (Halliwell 2006). L'excès d'EROs dans le corps peut endommager les composants cellulaires comme l'ADN, les lipides et les protéines (Johansen et al. 2005; Chedea et Pop 2019; Tai et Ascoli 2011; Dudonné et al. 2009; Mishra, Ojha, et Chaudhury 2012; Losada-Barreiro et Bravo-Díaz 2017; Rizzo et al. 2010).

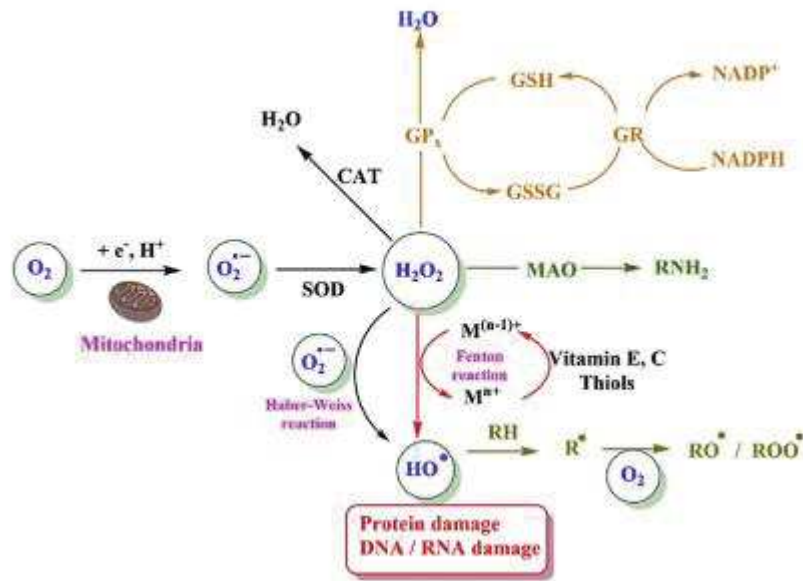


Figure 15. Illustration simplifiée des réactions impliquées dans la formation et l'élimination des ERO. GPx: glutathion peroxydase, GR: glutathion réductase, GSH: glutathion réduit; GSSG: disulfure de glutathion ou glutathion oxydé, SOD: superoxyde dismutase, CAT: catalase, MAO: monoamine oxydase, RH: lipide (Bhattacharya 2015; Losada-Barreiro et Bravo-Díaz 2017).

❖ Le stress oxydatif

Le stress oxydatif provient d'un déséquilibre entre les oxydants et les antioxydants en faveur des oxydants (Dudonné et al. 2009; Atig et al. 2017; Mirończuk-Chodakowska, Witkowska, et Zujko 2018). Il résulte de la surproduction de EROs et/ou de l'insuffisance des mécanismes de défense antioxydants (Berg, Youdim, et Riederer 2004; Wu, Kosten, et Zhang 2013). En fait, de nombreux composés cellulaires sont sensibles à ce déséquilibre, même léger, ce qui peut entraîner l'apparition de nombreuses maladies, y compris les maladies inflammatoires et chroniques telles que le cancer, le diabète de type 2, l'Alzheimer et les maladies cardiovasculaires (Chedea et Pop 2019; Khan 2012; Mishra, Ojha, et Chaudhury 2012). Un équilibre correct entre les deux phénomènes antioxydation – oxydation est nécessaire pour un système biologique sain (Dudonné et al. 2009; Chedea et Pop 2019).

3.1.2. Antioxydants

Les antioxydants sont des composés capables de réduire ou d'empêcher la destruction oxydative des composés biologiques (Balík et al. 2009). Ils protègent les systèmes biologiques en utilisant différents mécanismes préventifs en tant que première ligne de défense tels que le piégeage des radicaux libres, la rupture des réactions en chaîne initiées par les radicaux libres et au final la réparation ou l'élimination des structures endommagées (Rizzo et al. 2010; Mirończuk-Chodakowska, Witkowska, et Zujko 2018). Différents antioxydants sont impliqués pour maintenir l'équilibre du système biologique, y compris des antioxydants endogènes (enzymatiques et non enzymatiques), (Chedea et Pop 2019; Mirończuk-Chodakowska, Witkowska, et Zujko 2018; Nordberg et Arner, s. d.) et des antioxydants alimentaires (Chedea et Pop 2019). Les deux types d'antioxydants agissent en synergie afin de maintenir ou rétablir l'homéostasie redox (Zujko et al. 2012).

❖ Les antioxydants endogènes

Ce sont des produits du métabolisme du corps, ils peuvent être enzymatiques ou non enzymatiques et ils interviennent en première ligne de défense (défense préventive).

Antioxydants non enzymatiques

On trouve le glutathion, l'albumine, la bilirubine et l'acide urique (Chedea et Pop 2019; Wu, Kosten, et Zhang 2013).

Antioxydants enzymatiques

On trouve la superoxyde dismutase (SOD) (EC 1.15.1.1), la glutathion peroxydase (GPx) (EC 1.11.1.9), la catalase (CAT) (EC 1.11.1.6) (Chedea et Pop 2019; Wu, Kosten, et Zhang 2013; Steenvoorden et Beijersbergen van Henegouwen 1997), la glutathion réductase (GR) et les peroxyrédoxines (Prxs) (Mirończuk-Chodakowska, Witkowska, et Zujko 2018).

❖ Les antioxydants alimentaires

Les denrées alimentaires sont considérées comme des sources naturelles contenant différents composés antioxydants comme les caroténoïdes, les vitamines C et E et les polyphénols (Tableau 3) (Amarowicz et Pegg 2019; Marfella et al. 2014; Guo et al. 2017; G. Kaur et al. 2016).

Les polyphénols sont de puissants antioxydants naturellement présents dans le régime alimentaire, notamment dans les fruits, les légumes, les céréales et le thé (Chedea et Pop 2019; Losada-Barreiro et Bravo-Díaz 2017). Les polyphénols neutralisent les radicaux libres en cédant un électron ou un atome d'hydrogène pour réduire le taux d'oxydation en inhibant la formation des radicaux libres ou en les piégeant (Marfella et al. 2014). Le raisin est classé parmi les aliments les plus riches en composés phénoliques. L'activité antioxydante du raisin est liée directement à la concentration, la composition phénolique (Doshi, Adsule et Banerjee 2006), au nombre et à la position des groupements OH (Losada-Barreiro et Bravo-Díaz 2017).

Tableau 3. Les principaux composés antioxydants dans les aliments

Aliments	Principaux composés antioxydants	Références
Pomme	quercétine glycosides, procyanidines B2, acide chlorogénique, épicatechine et vitamine C	(K. W. Lee et al. 2003)
Abricot	acide néochlorogénique, l'acide chlorogénique, catéchine, epicatechin, rutine, quercétine 3- glucoside, acides hydroxycinnamiques, flavanols, flavonols, acide ascorbique	(Madrau et al. 2009)
Raisin	polyphénols	(Lutz et al. 2011)

Pamplemousse	acide gallique, β - carotène, acide protocatéchique, acide vanilique, acide caféique, acide coumarique, acide férulique, acide sinapique	(Marfella et al. 2014; Kaur et al. 2016; Gorinstein et al. 2004; Cömert et Gökmen 2018)
Kiwi	procyanidine B1, catéchine, épicatechine, acide caféique et ses dérivés, acide férulique et ces dérivés dérivés d'acide coumarique et le kaempférol	(Guo et al. 2017)
Mangue	α -tocophérol, acide ascorbique, caroténoïdes, flavonoïdes et mangéfirine.	(Li et al. 2014)
Agrumes	vitamine C et flavonoides	(Ramful et al. 2011; Kaur et al. 2016)
Salade	flavones, flavonols, anthocyanes, dérivés d'acide caféique	(Llorach et al. 2008)
Tomate	caroténoïdes, acide ascorbique,	(G. Kaur et al. 2016; Cömert et Gökmen 2018; Müller et al. 2016; George et al. 2004)

3.1.3. Détermination de la capacité antioxydante

Compte tenu de la complexité des processus d'oxydation et de la nature des antioxydants, avec des composants à la fois hydrophiles et hydrophobes, il existe plusieurs méthodes pour mesurer l'activité antioxydante (figure 16). Le plus souvent il faut combiner les réponses des différents tests pour avoir une indication sur la capacité antioxydante de l'échantillon (Popovici, Saykova, et Tylkowski 2009).

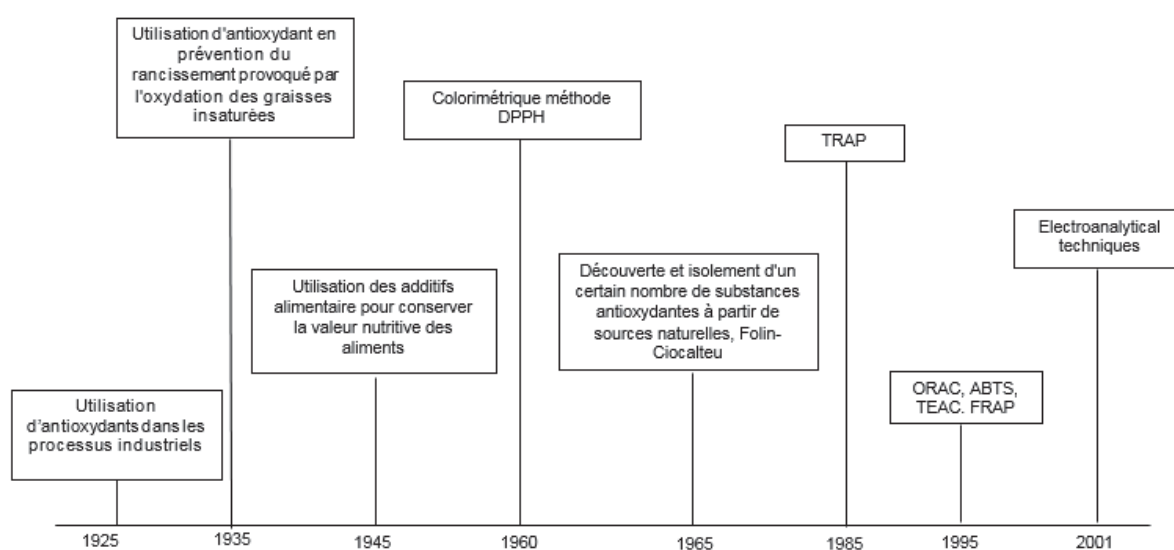


Figure 16. Chronologie des principaux développements de la recherche sur les antioxydants alimentaires (Cömert et Gökmen 2018).

3.1.3.1. Tests spectrophotométriques

Au cours de ces dernières décennies, une large gamme de tests spectrophotométriques a été développée afin de mesurer la teneur en polyphénols totaux et ainsi que la capacité antioxydante des aliments (Sethi et al. 2020; Floegel et al. 2011; Thaipong et al. 2006; El Rayess et al. 2014; Magalhães et al. 2014). Les tests colorimétriques les plus connus sont : le dosage de Folin-Ciocalteu, le test avec l'acide 2,2'-azino-bis(3-éthylbenzthiazoline-6-sulfonique) (ABTS) (Abramovič et al. 2015), le test avec le 1,1 -diphényl-2-picrylhydrazyle (DPPH) (Mishra, Ojha, et Chaudhury 2012;

Popovici, Saykova, et Tylkowski 2009; Danilewicz 2015; Abramovič et al. 2015) et la capacité de réduction ferrique du plasma (FRAP) (Thaipong et al. 2006; Dudonné et al. 2009).

Le test de **Folin-Ciocalteu** : il est largement utilisé et il donne une indication globale sur la teneur en composés phénoliques totaux (Yilmaz et al. 2015; Coklar 2017; Lingua et al. 2019; Ky et Teissedre 2015). Le réactif de Folin est un complexe de phosphotungstate et phosphomolybdate qui sont réduits par les composés phénoliques en condition alcaline (milieu réactif ajusté par du carbonate de sodium avec un pH proche de 10) produit une coloration bleue avec une absorbance maximale à 765 nm. Les résultats sont exprimés en équivalents d'acide gallique. Le réactif de Folin n'est pas spécifique aux composés phénoliques car il peut réagir avec d'autres composés comme l'acide ascorbique, le cuivre (Lorrain et al. 2013; Singleton, Orthofer, et Lamuela-Raventós 1999; Huang, Ou, et Prior 2005; Stevanato, Fabris, et Momo 2004; Danilewicz 2015), les sucres, les amines aromatiques (Prior, Wu, et Schaich 2005). Le test de Folin Ciocalteu est un test simple, pratique et reproductible (Huang, Ou, et Prior 2005; El Rayess et al. 2014; Magalhães et al. 2014).

Le test **ABTS** a été utilisé pour la première fois par Miller et Rice-Evans en 1993 puis il a été amélioré plus tard par (Re et al. 1999). Le radical ABTS[•] est généré par l'oxydation du persulfate de 2,2'-azinobis (acide 3-éthylbenzothiazoline-6-sulfonique) (Floegel et al. 2011). La capacité antioxydante est déterminée par la diminution de l'absorbance du radical ABTS[•] en présence de l'échantillon à tester après un temps fixe (4-6 min) à la longueur d'onde de 734 nm. Les résultats sont exprimés en équivalent de trolox ou en pourcentage de diminution de l'absorbance (Huang, Ou, et Prior 2005; Cano, Acosta, et Arnao 2000). L'ABTS est un test simple, rapide et peut être utilisé sur une large gamme de pH (Lemańska et al. 2001; Magalhães et al. 2014).

Le test **DPPH** est utilisé pour la première fois par Brand-Williams et ses collègues (Brand-Williams, Cuvelier, et Berset 1995). Il est basé sur la réduction du radical violet DPPH[•] en hydrazine jaune pâle par les antioxydants (Magalhães et al. 2014). Les polyphénols réduisent le radical avec une perte d'absorbance qui est suivie au cours

du temps (30 min) à 515 nm (Popovici, Saykova, et Tylkowski 2009; Katalinić et al. 2004). Les résultats sont exprimés soit en pourcentage de diminution de l'absorbance (Katalinić et al. 2004) soit en équivalent trolox. Le DPPH est un test rapide et simple ne nécessite pas beaucoup de réactifs, ce qui explique probablement son utilisation répandue dans la détermination de la capacité antioxydante (Magalhães et al. 2014; El Rayess et al. 2014).

Le test **FRAP** : ce test a été initialement développé par Benzie and Strain (Benzie et Strain 1996) pour mesurer le pouvoir réducteur dans le plasma. Ce test a été adapté par la suite aux antioxydants des végétaux (Ou et al. 2002; Gil et al. 2000; Proteggente et al. 2002). Il est différent des autres (DPPH et ABTS) car il n'y a pas de radicaux libres impliqués, il est basé sur la réduction du fer ferrique (Fe^{+3}) en fer ferreux (Fe^{+2}) (Floegel et al. 2011) et la puissance de chélation sur les ions ferreux (Fe^{2+}) (Sudan et al. 2014). L'absorbance est mesurée après 4 min à 593 nm. Les résultats sont exprimés par rapport à une solution standard de Fe (II) (Dudonné et al. 2009; Thaipong et al. 2006; Sethi et al. 2020). Le test FRAP est rapide, simple, peu coûteux et robuste et ne nécessite pas d'équipement spécialisé (Prior, Wu, et Schaich 2005; Danilewicz 2015).

Les inconvénients des méthodes spectrophotométriques

Les analyses spectrophotométriques peuvent conduire à :

- une surestimation des teneurs en polyphénols et de leur capacité antioxydante des échantillons due à l'interférence possible des molécules qui absorbent en UV (Danilewicz 2015; El Rayess et al. 2014; Magalhães et al. 2014).
- la consommation des solvants et le temps d'analyse (Lorrain et al. 2013; Danilewicz 2015).

D'autres méthodes simple, rapide, robuste et fiable a été développée pour déterminer la capacité antioxydante : ce sont les techniques électrochimiques (Lorrain et al. 2013).

3.1.3.2. Tests électrochimiques

Différentes méthodes électrochimiques sont utilisées pour la caractérisation et la quantification des polyphénols et de la capacité antioxydante : la chronoampérométrie, la voltammétrie à impulsion différentielle et la voltammétrie cyclique (Hoyos-Arbeláez, Vázquez, et Contreras-Calderón 2017) basées sur le fait que tous les composés phénoliques sont électrochimiquement actifs (Mark, Scholz, et Matysik 2012; Makhotkina et Kilmartin 2010; Arribas, Martínez-Fernández, et Chicharro 2012; Ricci et al. 2019; Zou et al. 2002; Orlandi et al. 2018).

La voltammétrie cyclique est la méthode électrochimique la plus utilisée pour la caractérisation des polyphénols et la détermination de la capacité antioxydante (Kilmartin, Zou, et Waterhouse 2001; 2002; Hoyos-Arbeláez, Vázquez, et Contreras-Calderón 2017). L'aire sous la courbe d'oxydation sur le voltammogramme représente la capacité antioxydante totale de l'échantillon (Rockenbach et al. 2011; José Jara-Palacios et al. 2017). La présence de pics à faible potentiel est corrélée à la présence des polyphénols à forte activité antioxydante, tandis que les composés à faible pouvoir antioxydant donnent des pics ayant un potentiel plus élevé (El Rayess et al. 2014; Hoyos-Arbeláez, Vázquez, et Contreras-Calderón 2017).

En effet, les ortho-diphénols facilement oxydés donnent un pic de potentiel faible autour de 400 mV, les anthocyanes produisent un pic à 650 mV, et les groupes fonctionnels les plus difficiles à oxyder produisent des pics à des potentiels plus élevés, permettant une discrimination entre ces types de molécules (Lorrain et al. 2013; Newair, Kilmartin, et Garcia 2018; Kilmartin, Zou, et Waterhouse 2002).

3.2. L'activité anti-inflammatoire

3.2.1. Inflammation

L'inflammation est une réponse de défense du système immunitaire aux agents pathogènes étrangers (Kishore et al. 2019; Riegsecker et al. 2013) notamment les dommages physiques, l'irradiation aux ultraviolets et l'invasion microbienne, afin d'éliminer les stimuli, les tissus endommagés et puis initier la guérison et la réparation

des tissus (Gautam et Jachak 2009; Mariathasan et Monack 2007). Les signes physiques de l'inflammation les plus évidents sont la douleur, la chaleur, la rougeur et l'enflure qui sont dues à l'augmentation du flux sanguin, de la vasodilatation, la libération des médiateurs intracellulaires et aux fuites de liquide (Ferrero-Miliani et al. 2006). Les macrophages, les neutrophiles et les phagocytes mononucléaires sont les principales cellules immunitaires impliquées dans la réponse inflammatoire.

Selon la réponse de l'action pour éradiquer l'agent étranger ou les tissus lésés, on distingue deux types d'inflammation :

Inflammation aiguë : elle est très rapide et commence quelques minutes après la lésion tissulaire visant à tuer les bactéries, virus ou les parasites sur les sites actifs. Elle implique l'association de protéines plasmatiques et la migration des fluides et des neutrophiles vers la région lésée (Heras et Hortelano 2009; Kishore et al. 2019). Pendant la réponse inflammatoire aiguë, les neutrophiles et les macrophages sont principalement stimulés (Ferrero-Miliani et al. 2006).

Inflammation chronique : ce processus est long et associé à la prolifération vasculaire, aux macrophages, à la fibrose et à la destruction des tissus (Kishore et al. 2019; Riegsecker et al. 2013). Les lymphocytes T, les plasmocytes et les macrophages sont les principaux propagateurs de l'inflammation chronique (Ferrero-Miliani et al. 2006; Riegsecker et al. 2013).

3.2.2. Marqueurs de l'inflammation

Les macrophages sont les principaux contributeurs à la réponse inflammatoire lors de la stimulation par des stimuli exogènes tels que les lipopolysaccharides (LPS) (Gautam et Jachak 2009; Han et al. 2017; Cheng et al. 2014; Wan et al. 2019) ou le muramyl dipeptide (Cheng et al. 2014). Ils secrètent des cytokines pro inflammatoires, notamment le facteur de nécrose tumorale α (TNF- α), l'interleukine-6 (IL-6) et l'IL-1 β , les médiateurs pro-inflammatoires dont l'oxyde nitrique (NO), la prostaglandine E2 (PGE2) et les espèces réactives de l'oxygène (ROS) (Du et al. 2018; Q. Xu et al. 2017) et les protéines inflammatoires telles que l'oxyde nitrique inducible synthase (iNOS) et cyclooxygénase-2 (COX-2). L'inhibition de la production des marqueurs

d'inflammation est le mécanisme clé dans le contrôle de l'inflammation (Q. Xu et al. 2017; Bak et al. 2013).

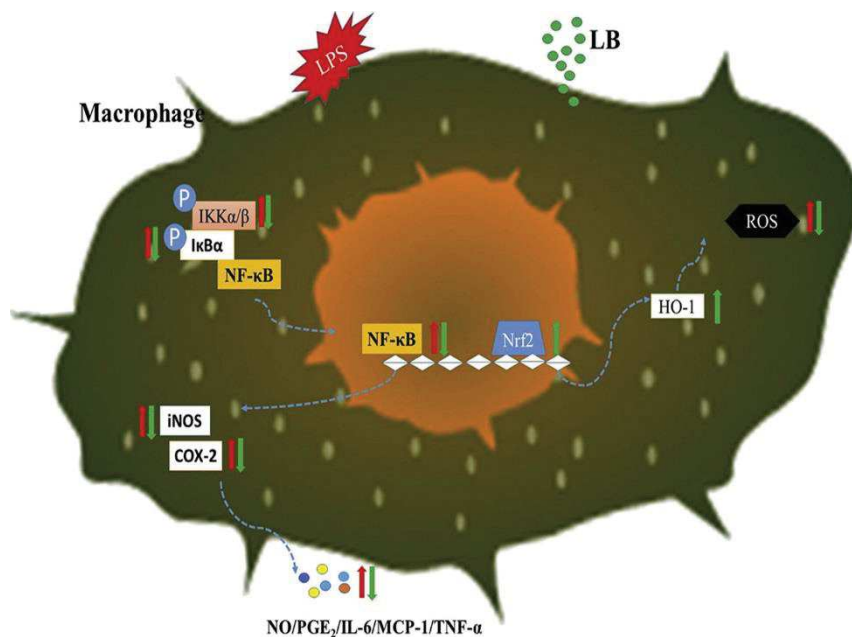


Figure 17. Stimulation des macrophages RAW 264.7 par les LPS (Linghu et al. 2020)

3.2.3. Complications de l'inflammation

L'inflammation chronique provoque des troubles au niveau des tissus sains conduisant à plusieurs maladies (Kishore et al. 2019; Singh, Akhtar, et Haqqi 2010) comme l'asthme, le vieillissement, l'athérosclérose, le trouble d'immunodéficience acquise (SIDA), l'athérosclérose, la goutte, le diabète (Kishore et al. 2019), la maladie d'Alzheimer, la maladie de Parkinson (Shao et al. 2013), le cancer et l'insuffisance cardiaque (Bak et al. 2013).

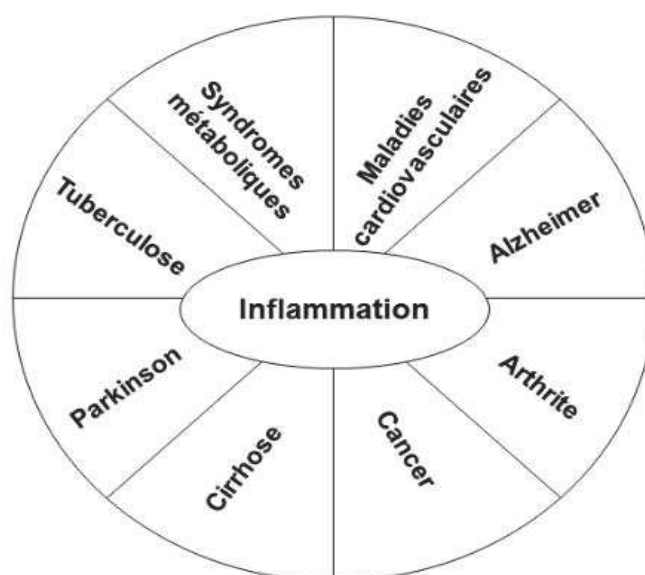


Figure 18. Troubles liés à l'inflammation (Kishore et al. 2019)

Plusieurs classes de médicaments tels que les anti-inflammatoires non stéroïdiens (AINS) et les corticostéroïdes sont utilisés pour traiter les troubles inflammatoires. Ces médicaments possèdent des effets indésirables tels que l'hypertension, l'hyperglycémie, faiblesse musculaire, ostéoporose et le diabète (Gautam et Jachak 2009). De nombreuses recherches ont montré que les composés phytochimiques tels que les polyphénols ont un effet anti-inflammatoire et peuvent être utilisés comme une alternative aux médicaments synthétiques pour la prévention et le traitement des troubles inflammatoires (Du et al. 2018; Riegsecker et al. 2013; Rivière et al. 2014; Cheng et al. 2014; Lin et Li 2018; Terra et al. 2007; Bak et al. 2013; Fernández-Fernández et al. 2019).

3.3. L'activité antidiabétique

3.3.1. Physiopathologie du diabète

Le diabète est une maladie métabolique chronique caractérisée par une augmentation du taux de sucre plasmatique causé par des altérations de la sécrétion d'insuline, par l'inefficacité de l'insuline ou bien les deux (Dhameja et Gupta 2019; C. M. M. Santos, Freitas, et Fernandes 2018; Nasab et al. 2020; Abbas et al. 2019; Maritim, Sanders, et Watkins 2003; Kadouh et al. 2016). Le diabète augmente avec

des taux alarmants dans le monde (Figure 19). Ainsi en 2000, le nombre de cas atteints du diabète était de 151 millions et ce chiffre a très fortement augmenté pour atteindre les 463 millions en 2019 (International Diabetes Federation, IDF diabetes atlas).

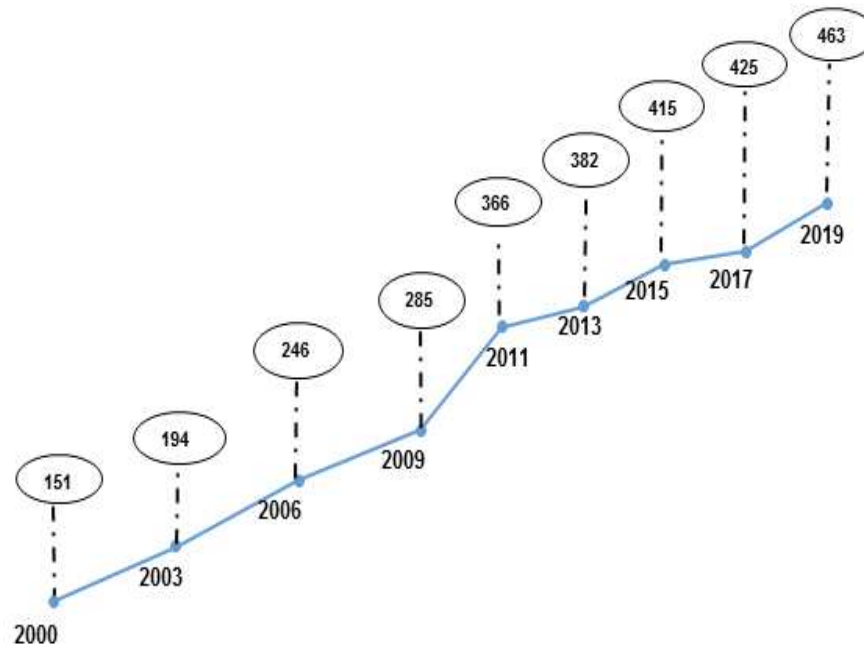


Figure 19. Estimations de la prévalence mondiale du diabète chez les personnes entre 20 et 79 ans (en millions).

Le diabète est divisé en trois catégories ; le diabète de type 1, de type 2 et le diabète gestationnel (Dhameja et Gupta 2019; Zimmet, Alberti, et Shaw 2001; Tesauro et Mazzotta 2020; C. M. M. Santos, Freitas, et Fernandes 2018).

Le diabète de type 1 : il est appelé aussi insulino-dépendant et représente entre 5 à 10 % des cas de diabète (García-Pérez, Kasangana, et Stevanovic 2017). Il est dû à la destruction auto-immune des cellules bêta pancréatiques par des facteurs génétiques et environnementaux, conduisant à une carence ou une insuffisance de la production d'insuline endogène (Figure 20) (Zimmet, Alberti, et Shaw 2001; Tesauro et Mazzotta 2020; J. Santos et al. 2014; García-Pérez, Kasangana, et Stevanovic 2017). Le traitement principal de ce type de diabète consiste à faire des injections d'insuline tous les jours (Nasab et al. 2020; Shukla et al. 2011).

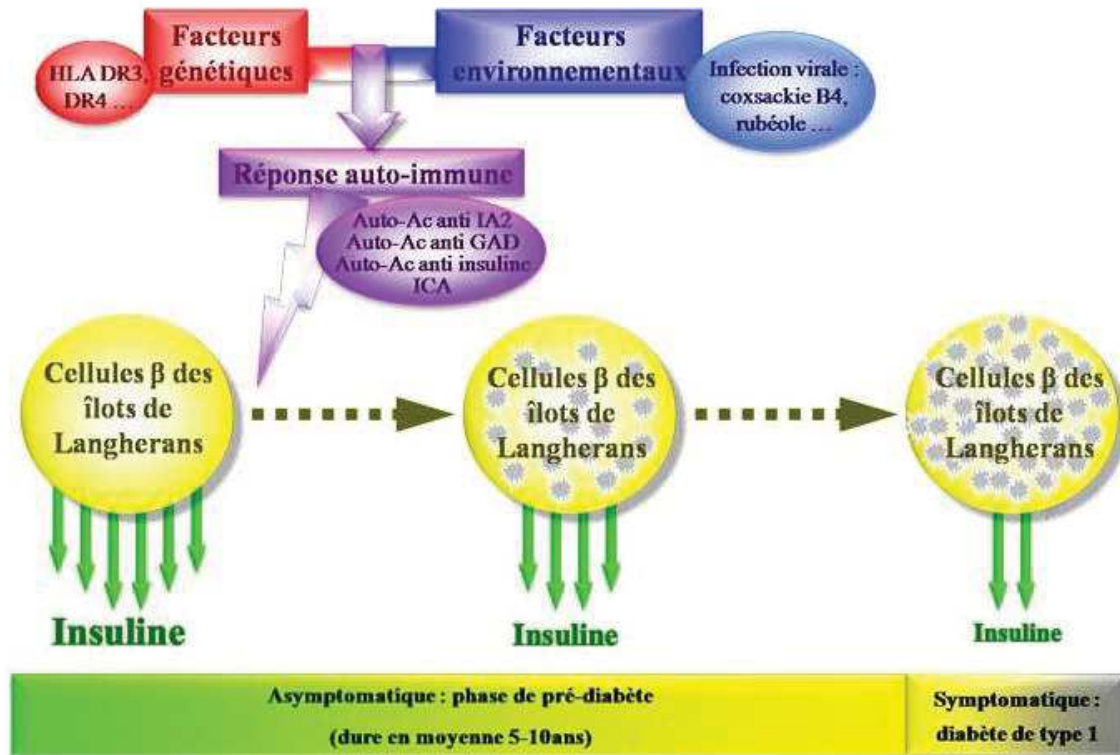


Figure 20. Physiopathologie du diabète de type 1 (https://www.memobio.fr/html/bioc/bi_did_ph.html)

Le diabète de type 2 : il est appelé aussi non insulino-dépendant et c'est la forme la plus courante du diabète (80 % à 90 % des cas (Dhameja et Gupta 2019)). Le diabète de type 2 survient lorsque le corps ne peut pas utiliser correctement l'insuline. Ceci est dû à une perte progressive de la sécrétion et/ou de la sensibilité à l'insuline (Zimmet, Alberti, et Shaw 2001; Tesauro et Mazzotta 2020; J. Santos et al. 2014). Ce type de diabète peut être géré en modifiant le mode de vie, le régime alimentaire et en prenant des médicaments oraux (Nasab et al. 2020). Il y a une forte prédisposition génétique au diabète de type 2. L'âge, l'obésité et la sédentarité sont également des facteurs de risque (Figure 21) (Shukla et al. 2011; Tesauro et Mazzotta 2020; Ezuruike et Prieto 2014). Le type 2 affecte principalement les personnes de plus de 40 ans (Shukla et al. 2011). Les deux types de diabète sont précédés d'une longue phase asymptomatique, appelé prédiabète, qui se caractérise par une légère hyperglycémie et une diminution précoce de la capacité de sécrétion d'insuline (Inzucchi 2012).

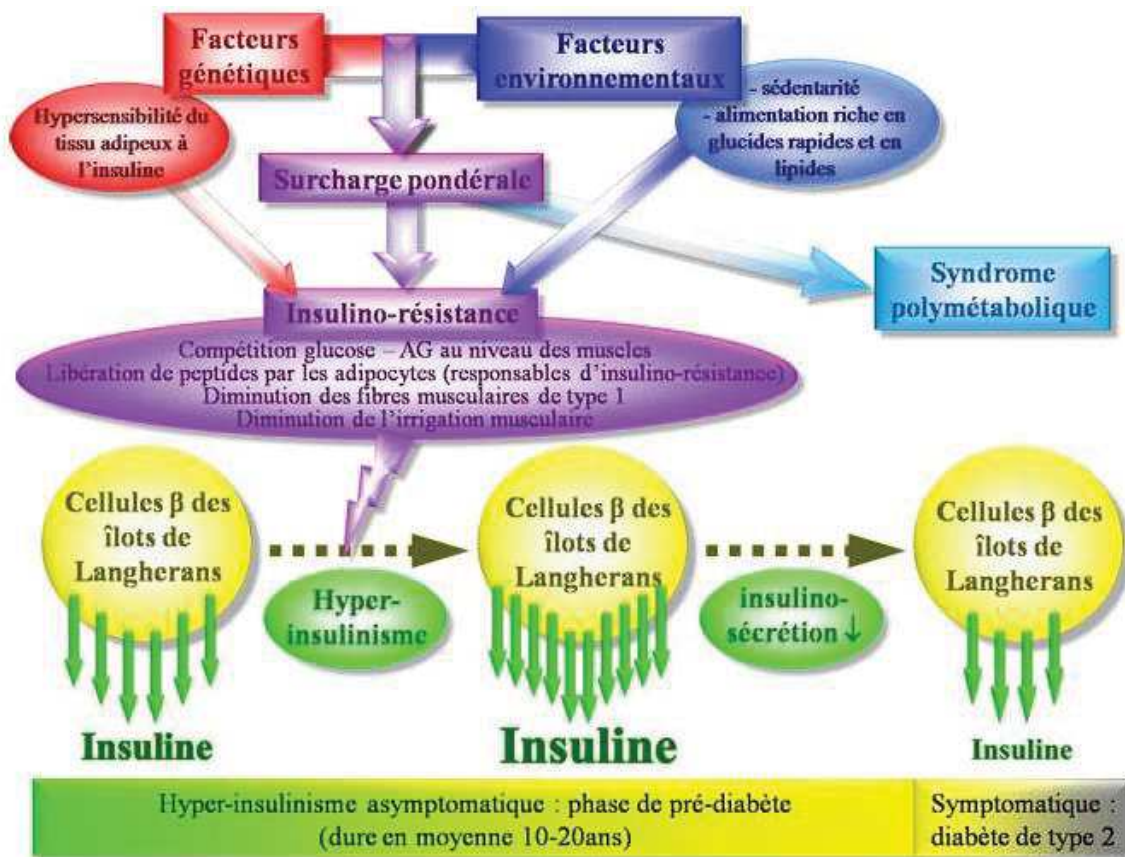


Figure 21. Physiopathologie du diabète de type 2 (https://www.memobio.fr/html/bioc/bi_dni_ph.html)

Diabète gestationnel : il survient pendant le deuxième ou le troisième trimestre de la grossesse (Tesauro et Mazzotta 2020). Il se développe lorsque les hormones de grossesses bloquent l'action de l'insuline et il y a un risque important de mortalité pour la mère ou le bébé (Shukla et al. 2011; C. M. M. Santos, Freitas, et Fernandes 2018).

3.3.2. Diagnostiques, symptômes et complications du diabète

❖ Symptômes

Selon l'OMS (https://www.who.int/diabetes/action_online/basics/fr/index1.html), les symptômes du diabète sont :

- la sensation de soif, de faim, l'excrétion excessive d'urine et un amaigrissement inexplicé.

- un engourdissement des extrémités, des douleurs dans les pieds (sensations de gêne) et une vision floue.
- des infections récurrentes ou graves.
- une perte de conscience, des nausées/vomissements sévères (acidocétose) ou le coma. L'acidose est plus fréquente en cas de diabète type 1 que de type 2.

❖ Diagnostics

L'hyperglycémie est diagnostiquée cliniquement par la mesure du taux de glucose dans le sang, qu'elle soit à jeun ou spontanément à tout moment de la journée (Figure 22). L'HbA1c, est considérée exclusivement comme l'élément de surveillance du diabète depuis 2009 (Tenenbaum et al. 2018).

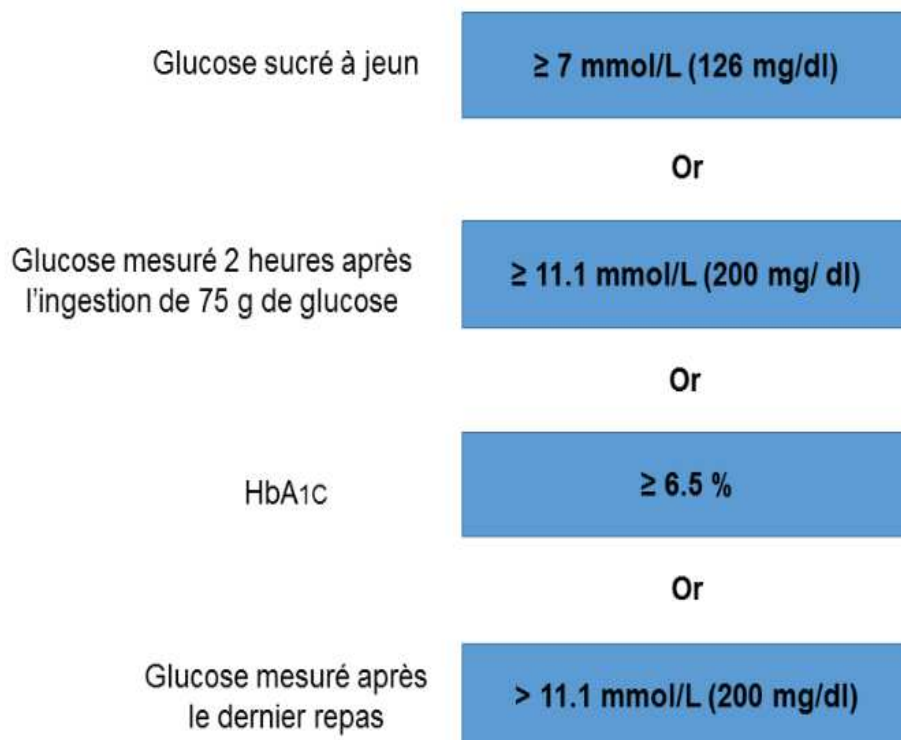


Figure 22. Critères de diagnostic du diabète (Federation International Diabetes IDF Diabetes Atlas Ninth. 2019).

❖ Complications

Le diabète est généralement silencieux à ses débuts et, à long terme, il peut entraîner des complications dues à la libération des radicaux libres qui jouent un rôle important dans l'apparition et la progression des plusieurs maladies telles que les maladies rénales, les maladies du système nerveux, les maladies cardiaques et la rétinopathie (Dhameja et Gupta 2019; Nasab et al. 2020; Abbas et al. 2019; Buron et Thauinat 2020; García-Pérez, Kasangana, et Stevanovic 2017).

3.3.3. Traitements pharmacologiques

L'une des stratégies thérapeutiques les plus importantes contre le diabète est de diminuer le taux de glucose postprandial par l'inhibition de la dégradation enzymatique des polysaccharides sans effet direct sur la sécrétion d'insuline (Nasab et al. 2020; Di Stefano, Oliviero, et Udenigwe 2018). Par conséquent, l'inhibition des enzymes α -amylase et α -glucosidase est une approche efficace pour gérer l'index glycémique indépendamment de l'insuline (Figure 23) (Nasab et al. 2020; Dhameja et Gupta 2019; Abbas et al. 2019). Ces inhibiteurs sont utilisés en monothérapie dans les états diabétiques légers et sont utilisés en association avec l'insuline dans le diabète sévère (Dhameja et Gupta 2019).

L' α -amylase (EC 3.2.1.1): c'est une enzyme qui appartient à la classe des hydrolases. Les α -amylases salivaires et pancréatiques hydrolysent l'amidon en rompant les liaisons glycosidiques α -1,4 pour produire du maltose et d'autres oligosaccharides qui sont ensuite hydrolysés en glucose par l' α -glucosidase situé dans la membrane de surface en brosse des cellules intestinales (R. Kaur, Kaur, et Gupta 2014; Nasab et al. 2020; C. M. M. Santos, Freitas, et Fernandes 2018; Nyambe-Silavwe et al. 2015; Di Stefano, Oliviero, et Udenigwe 2018). Le glucose est absorbé par l'intestin et provoque l'hyperglycémie (Nasab et al. 2020; C. M. M. Santos, Freitas, et Fernandes 2018).

L' α -glucosidase (EC 3.2.1.20): c'est une enzyme qui catalyse le clivage des oligosaccharides et des disaccharides en monosaccharides lors de la dernière étape

de la digestion des glucides et qui augmente ainsi la concentration de glucose dans le corps (Abbas et al. 2019; Nasab et al. 2020; C. M. M. Santos, Freitas, et Fernandes 2018; Di Stefano, Oliviero, et Udenigwe 2018; Nyambe-Silavwe et al. 2015).

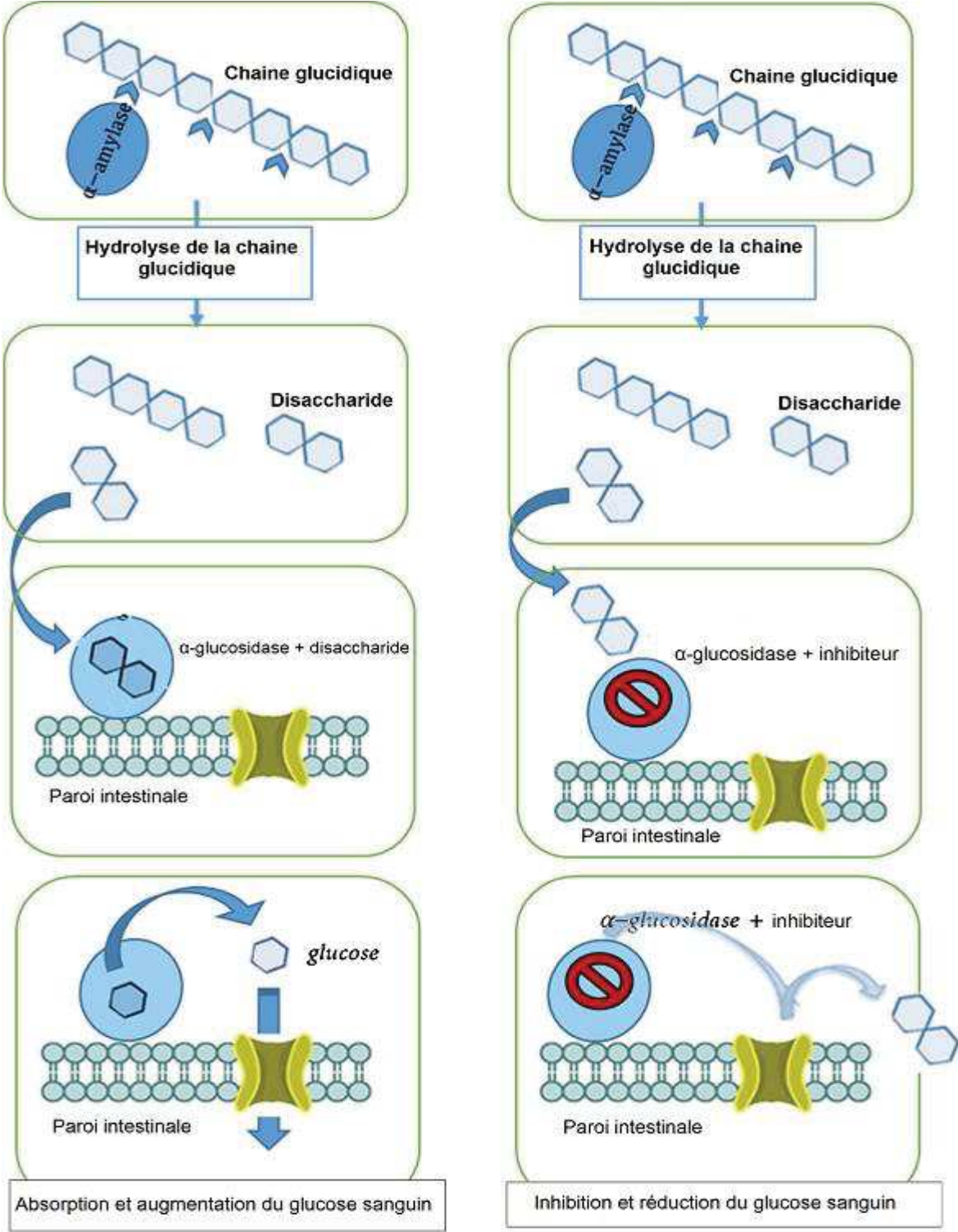


Figure 23. Réduction de la glycémie par l'inhibition de l'alpha glucosidase (Nasab et al. 2020)

Actuellement, quatre inhibiteurs de l' α -glucosidase sont utilisés en thérapie : l'acarbose, le miglitol, la voglibose et l'émiglitate dont l'acarbose est le premier inhibiteur introduit sur le marché dans les années 1990. L'utilisation de ces inhibiteurs est associée à des effets secondaires tels que les flatulences, les douleurs abdominales, la diarrhée et les nausées (C. M. M. Santos, Freitas, et Fernandes 2018; Nasab et al. 2020; Dhameja et Gupta 2019). Des aliments fonctionnels pourraient être développés contenant des composés capables d'inhiber l' α -amylase et l' α -glucosidase, une action de type acarbose, mais sans effet secondaire (Nyambe-Silavwe et al. 2015).

3.3.4. Traitements par les polyphénols

Il a été rapporté dans la littérature que certains extraits de plantes peuvent exercer une activité bénéfique pour la santé humaine en inhibant les activités des enzymes d'hydrolyse des glucides complexes. Les principaux constituants actifs de ces extraits de plantes sont des composés phénoliques tels que les flavonoïdes (Di Stefano, Oliviero, et Udenigwe 2018; Sun et al. 2016; Ibrahim et al. 2017; Abbas et al. 2019). L'activité inhibitrice dépend de la substitution de groupes hydroxyles qui peuvent former des liaisons hydrogènes avec l'enzyme (Gutiérrez-Grijalva et al. 2019) et au nombre des oligomères (les oligomères et les polymères ont plus d'activité en raison de leur grand nombre de sites de liaison avec les enzymes) (Di Stefano, Oliviero, et Udenigwe 2018).

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Chapitre 2

Les stilbènes du raisin et du vin et leurs propriétés anti-obésité

Les objectifs de ce chapitre consistent à faire une étude bibliographique sur :

- les stilbènes présents dans les pellicules de raisin et leurs concentrations.
- les facteurs qui impactent la synthèse et l'accumulation des stilbènes dans les pellicules de raisin.
- les stilbènes présents dans le vin et leurs concentrations.
- les facteurs qui influencent la concentration des stilbènes dans le vin.
- les méthodes analytiques utilisées pour la détection et la quantification des stilbènes dans le raisin et le vin
- les effets anti-obésité *in vitro* et *in vivo* des stilbènes et les mécanismes moléculaires associés.

Cette partie a fait l'objet d'une revue, soumise dans le journal *Trends in Food Science and Technology*

ET ci-après présentée.

Stilbenes in grape berries and wine and their potential role as anti-obesity agents: a review

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Background

Stilbenes are a group of naturally occurring phenolic compounds. These compounds are synthesized in response of biotic or abiotic stress situations. The major dietary sources of stilbenes are grape berries and wine. To accurately identify and quantify these compounds, the research community has undertaken considerable efforts to optimise samples preparations and analytical methods. In addition, stilbenes are well-known to possess a wide range of biological activities for human health. One of the most recent and promising properties demonstrated by stilbenes are their anti-obesity effects.

Scope and Approach

The principal objectives of this review to address and discuss about i) stilbenes in grape berries and wine, the factors that can modulate stilbenes concentrations and the most efficient sample preparation techniques and analytical methods used for their detection; ii) the most important investigations related to the *in vitro* and *in vivo* anti-obesity effects of grape and wine stilbenes and the associated molecular mechanisms.

Key Findings and Conclusions

Stilbene concentration in grape and wines can vary substantially. The composition of stilbenes in red wine is much more complex than in white wine. Until today, more than

30 stilbenes have been identified in grapes and wines. The liquid chromatography coupled to mass spectrometry is the most efficient method to investigate stilbene content. Regarding anti-obesity properties of stilbenes, a great number of *in vitro* and *in vivo* studies have allowed to demonstrate not only the positive implications of these bioactives but also the underlying mechanisms of the observed effects.

Keywords: stilbenes; resveratrol; grape, wine; *Vitis vinifera*; obesity

Abbreviation list

ACC: acetylCoA carboxylase

ACO: acyl-coenzyme A oxidase

aP2: adipocyte protein 2

ATAD3: ATPase family AAA Domain-containing protein 3

ATGL: adipose triglyceride lipase

BAT: brown adipose tissue

Bmp2: bone morphogenetic protein 2

BSTFA: bis(trimethylsilyl)trifluoroacetamide

CE: capillary electrophoresis

C/EBPs: CCAAT/enhancer binding proteins

CHS: chalcone synthase

CLA: conjugated linoleic acid

COX: cyclooxygenase

CPT-1: carnitine palmitoyltransferase 1

CRP: C-reactive protein

Cyc-D: cyclin D

DAD: diode array detector

Ddit3: DNA-damage inducible transcript 3

DLLME: dispersive liquid-liquid microextraction

ERK: extracellular receptor kinase

FABP4: fatty acid binding protein 4

FAS: fatty acid synthase

Fgf10: fibroblast growth factor 10

Fiaf: fasting-induced adipose factor

FLRD: fluorescence detector

FW: fresh weight

GC: gas chromatography

G6PDH: glucose-6-phosphate dehydrogenase

GLUT4: glucose transporter 4

HFCSD: high fructose corn syrup diet

HFD: high fat diet

HO-1: heme-oxygenase 1

HPD: high protein diet

HPLC: high performance liquid chromatography

HSL: hormone-sensitive lipase

IFN: interferon

IL: interleukin

IR: insulin receptor

KLF9: Kruppel-like factor 9

KR: β -ketoacyl reductase

LDL: low density lipoprotein

LPL: lipoprotein lipase

LXR α : liver X Receptor alpha

MS: mass spectrometry

MCP-1: monocyte chemoattractant protein 1

ME: malic enzyme

MEPS: microextraction by packed sorbents

Mfn2: mitofusin 2

miRNA: microRNA

MRM: multiple reaction monitoring

MS: mass spectrometry

mTOR: mammalian target of rapamycin

MTTP: microsomal triglyceride transfer protein

NF-KB: nuclear Factor Kappa B

Nrf1: nuclear respiratory factor 1

PDA: photodiode array detector

PDE3B: phosphodiesterase 3B

PKA: protein kinase A

PKC δ : Protein Kinase C delta

PPAR γ : peroxisome proliferator-activated receptor gamma

PRDM16: PR domain-containing 16

PVPP: polyvinylpolypyrrolidone

QqQ: triple quadrupole

QTOF: quadrupole time-of-flight

Rip 140: receptor interacting protein 140

ROS: reactive oxygen species

SBSE: stir-bar sorptive extraction

SCD-1: stearyl-CoA desaturase-1

SD: standard diet

SIRT1-AMPK-FOXO1: sirtuin 1-AMP-activated protein kinase-Forkhead box protein O1

SPE: solid phase microextraction

SPME: solid phase microextraction

SREBP1: sterol regulatory element binding transcription factor 1

STS: stilbene synthase

TG: triglycerides

TLR: Toll-like receptor

TNF- α : tumor necrosis factor alpha

UCP-1: uncoupling protein 1

UV: ultraviolet

WAT: white adipose tissue

WHO: world health organization

Highlights

- Grape berries and wines are among the major sources of stilbenes in human nutrition
- Red wines contain more complex stilbenes than white wines
- Stilbenes could reduce obesity by regulating different pathways
- Some stilbenes show better anti-obesity activities than resveratrol
- Combination of stilbenes with others polyphenols give promising results

Introduction

Stilbenes (1,2-diphenylethylene) are phenolic compounds derived from the general phenylalanine pathway (**Figure 1**). Among the commonly identified stilbenes, resveratrol is the most popular compound (Vang et al., 2011). The structural unit is constituted by two phenyl rings linked together by an ethylene bridge forming a C6-C2-C6 chain. This double bond allows stilbenes to exist in the *trans* (E) and in the *cis* form (Z) (Rivière et al., 2012). The aromatic rings could be substituted by different functional groups such as hydroxyl, methoxyl, prenyl or geranyl groups. Moreover, monomeric units can also be coupled, leading to the formation of oligomers. Over a thousand of natural stilbenes have been described in the literature. Despite this chemical diversity, only a limited number of plant families produce these secondary metabolites, such as Polygonaceae, Cyperaceae, Pinaceae or Vitaceae (Rivière et al., 2012). Furthermore, stilbenes are considered as phytoalexins since they are associated with the resistance of plants to diseases and their synthesis is often a response to an attack by phytopathogenic agents and other stress factors like UV irradiation, ozone, heavy metal ions, injury or frost (Błaszczuk et al., 2019; Langcake and Pryce, 1977).

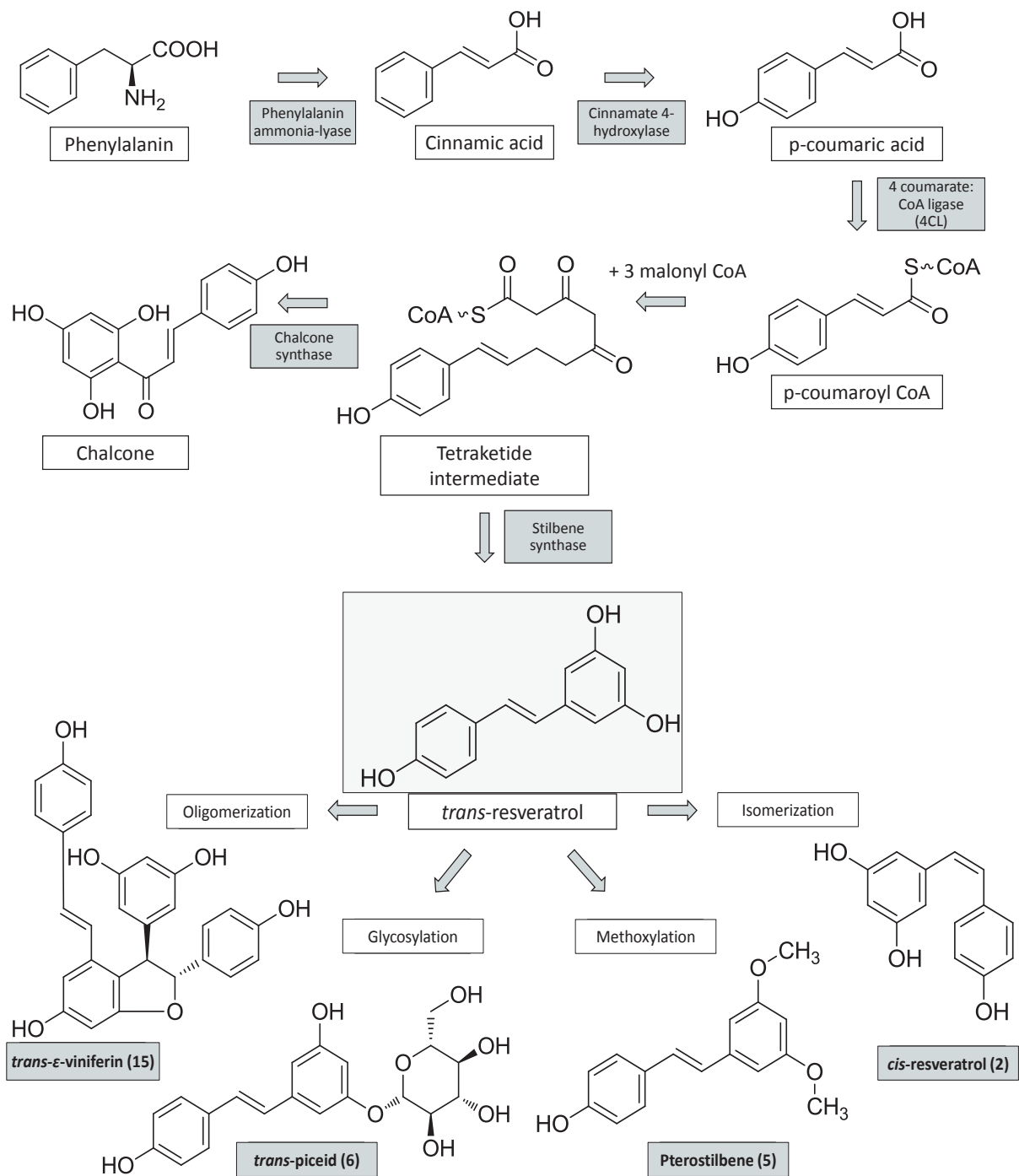
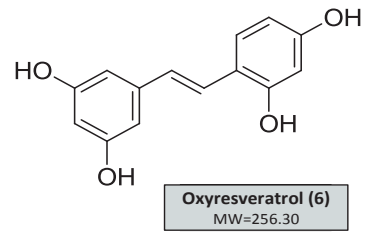
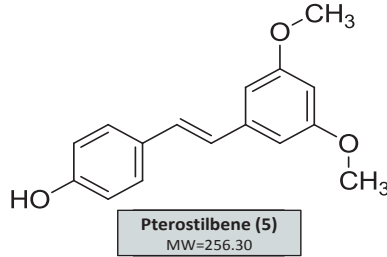
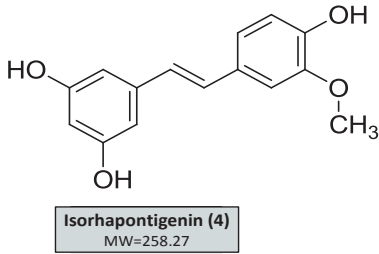
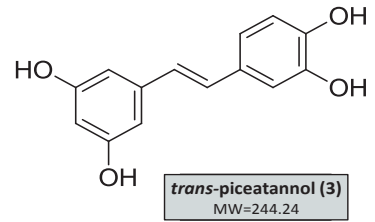
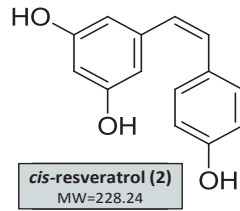
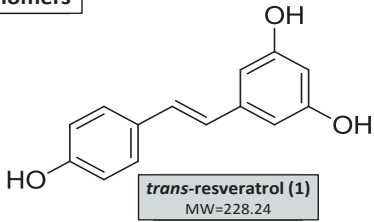


Figure 1. Biosynthesis of trans-resveratrol and its chemical diversification pathways (Chong et al., 2009)

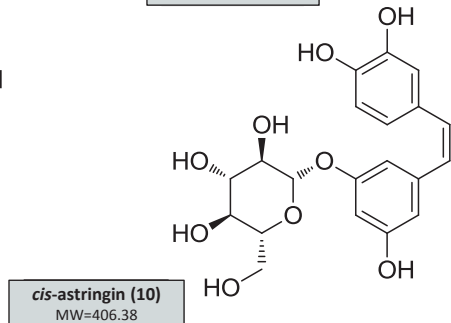
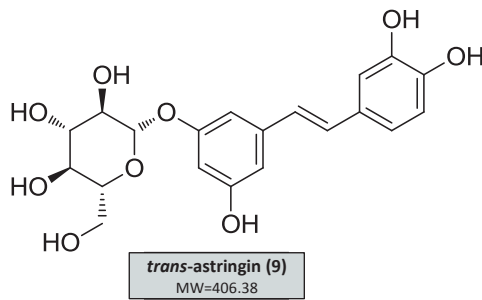
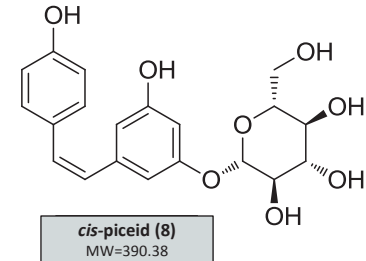
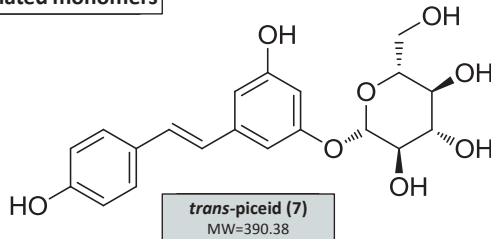
Relating to dietary sources, grapes and red wine are among the major sources of stilbenes in human nutrition and especially in European countries (Guerrero et al., 2009; Weiskirchen and Weiskirchen, 2016). However, it should be noted that stilbenes are also present in minor quantities in other foodstuffs such as peanut, pistachio, almonds, berries, banana, pineapple, apple, peach, passion fruit or dark chocolate (Neveu et al., 2010; Weiskirchen and Weiskirchen, 2016). Focusing only on grapes berries and wines, it is noteworthy that stilbene amounts can be extremely variable (Adrian et al., 2000; Guerrero et al., 2016; Hasan and Bae, 2017; Roldán et al., 2003; Vitrac et al., 2005). Several factors could influence their quantities in grapes such as the stage of ripening, the grape varieties or various external stimuli. Duration of maceration, yeast activity, and winemaking processes are also important factors that contribute to the final stilbene amounts described in wines.

For a long time, stilbene analyses in grape berries and wines were limited to resveratrol and piceid (Lamuela-Raventós et al., 1995; Mattivi et al., 1995). Today, thanks to the analytical methods such as ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC–MS/MS), more than thirty-three stilbenes have been identified and quantified in grape berries and wines (**Figure 2**). In addition, it must be emphasized that not only the selection of the most adapted analytical method but also the sample preparation techniques are crucial to have reliable and accurate results.

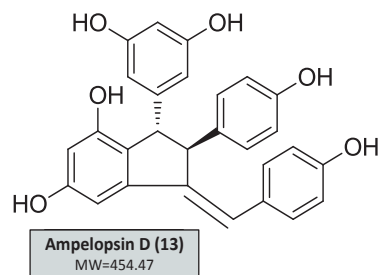
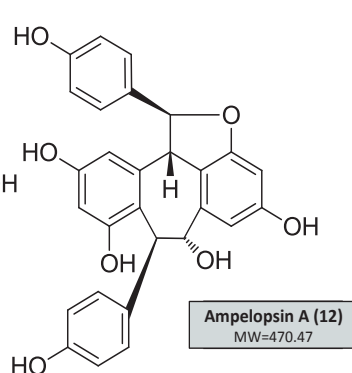
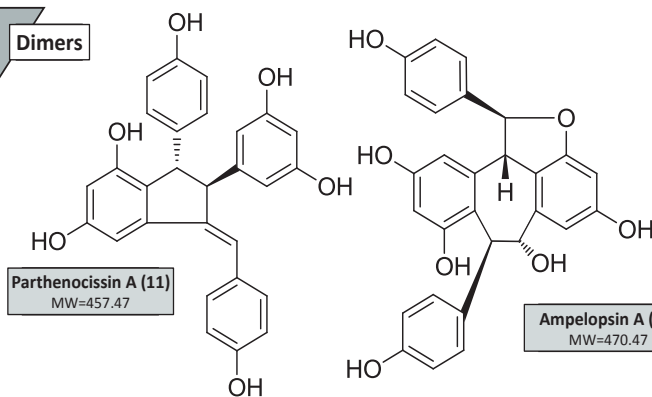
Monomers

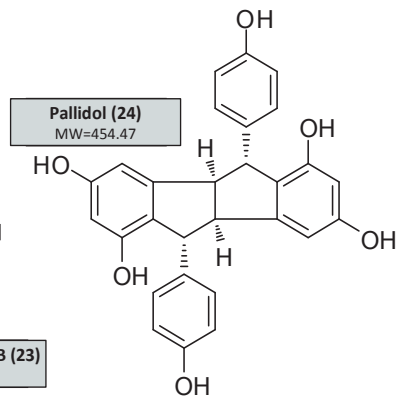
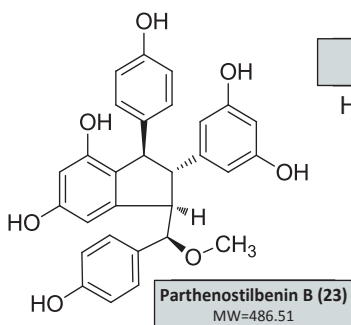
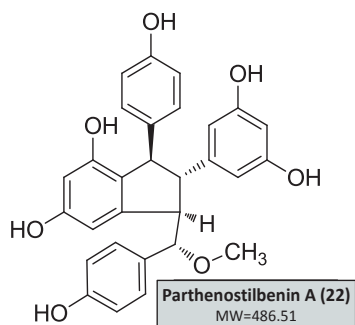
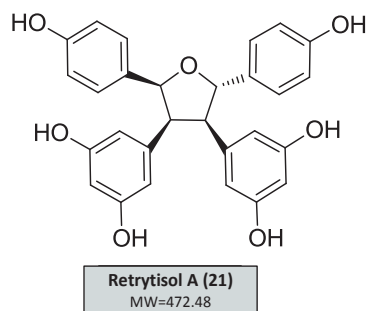
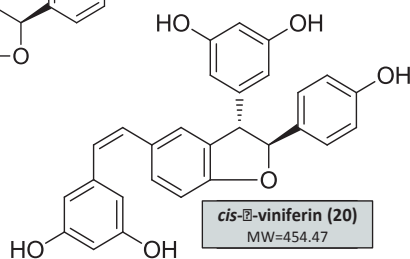
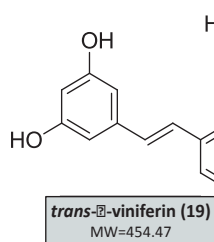
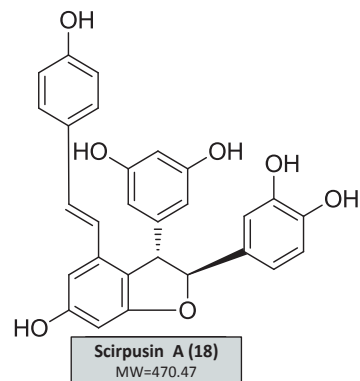
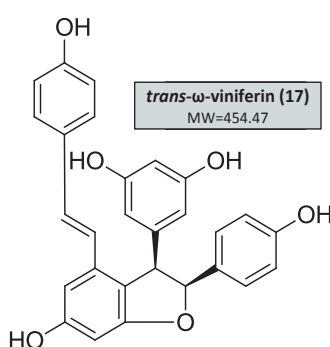
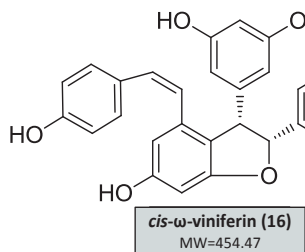
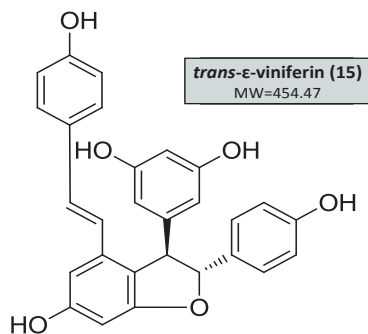
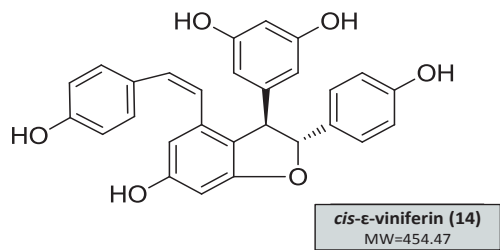


Glycosylated monomers

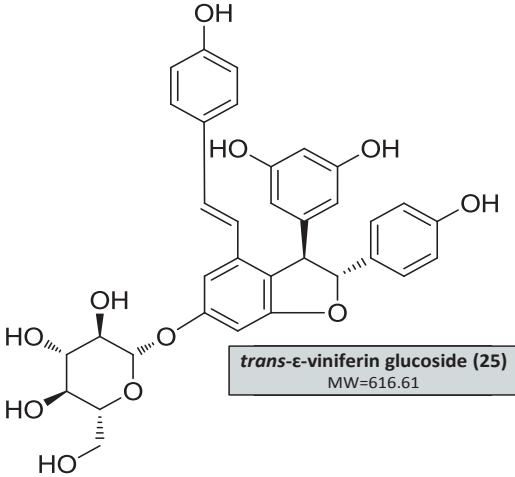


Dimers

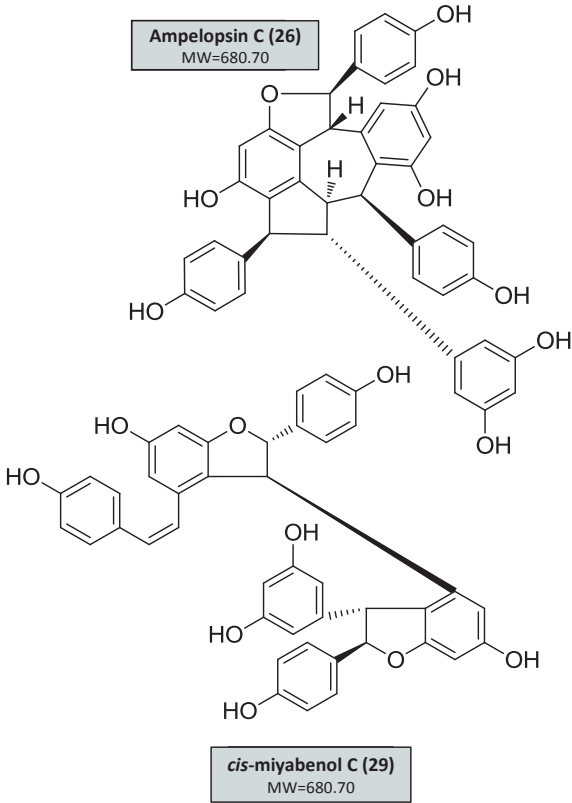
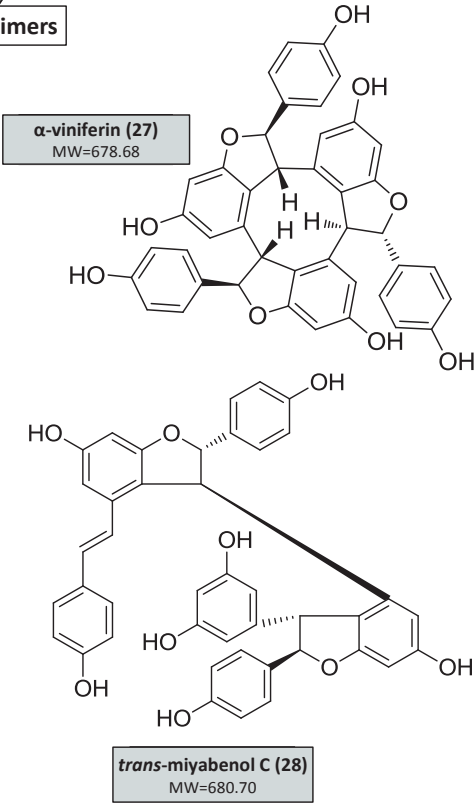




Glycosylated dimer



Trimers



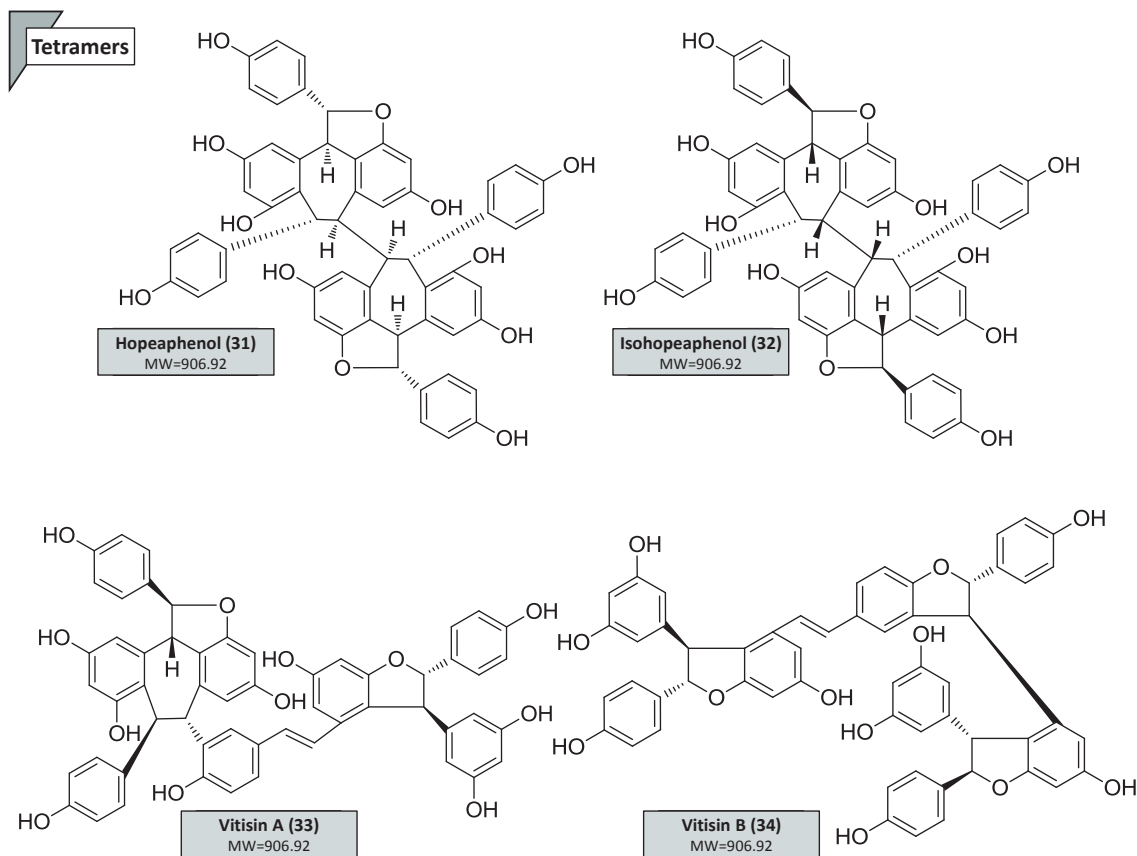


Figure 2. Main stilbenes identified in grape berries and win

Stilbenes have demonstrated to possess a great range of biological activities potentially beneficial for human health such as neuroprotective, antioxidant and antitumor effects (Guerrero et al., 2009; Vang et al., 2011; Weiskirchen and Weiskirchen, 2016). Among the more recent research lines, stilbenes are gaining considerable interest as potential anti-obesity agents. Obesity is the most common nutritional disorder in the world. According to the World Health Organization (WHO), obesity is defined as an abnormal or excessive fat accumulation that may impact health. Prevalence data estimate that 650 millions of adults were obese in 2016, representing about 13% of the world adult population. Moreover, alarming figures are presented regarding young population. In fact, more than 124 millions of children and adolescents were obese in 2016 (WHO, 2018, <http://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight>). It is well known that the fundamental cause of obesity is an energy imbalance between calories intake and loss, as a consequence globally, of a high intake of energy-fat foods and a sedentary style of life. When a positive energy imbalance occurs, triglycerides (TG) are accumulated in adipocytes producing an increase in the number of adipocyte (hyperplasia) or an increase of its size (hypertrophy) (Hausman et al., 2001). Additionally, obesity can also bring a potential risk to develop cardiovascular diseases, diabetes and some types of cancer, endometrial, breast, ovarian, prostate, liver, gallbladder, kidney, and colon, among others (Gallagher and LeRoith, 2015). Due to the increase of the prevalence and the associated complications, several global government policies, laws and regulations have been developed to halt and reverse the obesity epidemic. The first solution to stem obesity is focused in a change of lifestyle by decreasing the energy/fat/sugar intake and increasing physical activity. However, the implementation and effectiveness of these recommendations are usually unsatisfactory. It is therefore that research

community tries to find anti-obesity bioactive molecules, as stilbenes, that can be combined with other recommendations and treatments in order to ameliorate the results.

It has been shown that stilbenes may reduce obesity by regulating different pathways related to fat metabolism as adipogenesis, lipogenesis, lipolysis and thermogenesis (Chou et al., 2018; Fernández-Quintela et al., 2017). Resveratrol has been the most studied bioactive compound, but a considerable number of works are already indicating that other stilbenes are promising anti-obesity agents and may even be more potent than resveratrol.

For all above explained, the aim of this review is to address and discuss about:

i) stilbene compounds in grape berries and wines, the principal factors that can modulate stilbenes concentrations and the most efficient sample preparation techniques and analytical methods used for their detection;

ii) the most important investigations related to the *in vitro* and *in vivo* anti-obesity effects of grape and wine stilbenes and the associated molecular mechanisms.

Stilbenes content in grape berries and wine

Grape berries stilbenes

In grape berries, stilbenes as other polyphenols are mainly concentrated in skins (Babazadeh et al., 2017). They present a great variability in composition and content depending on different biotic and abiotic factors including grape variety, stage of ripening, viticultural factors and practices (Bavaresco et al., 2012; Błaszczuk et al., 2019). For example, according to Adrian et al. (Adrian et al., 2000), resveratrol concentration was 2 to 30 fold higher in berries induced with UV light compared to non-induced ones.

The stilbene content offers sharp contrasts due to many potential external stimuli (Vincenzi et al., 2013). Red varieties seem to present higher stilbene content than white ones (Guerrero et al., 2010; Viñas et al., 2011). Concerning monomers, glucosides (piceid and astringin) are the main compounds identified in grape berries (**Table 1**). The piceid (mean 1.36 mg/kg, *cis*- and *trans*-isomers) is the main compound followed by astringin (mean 0.83 mg/kg), and resveratrol (mean 0.68 mg/kg). Both isomeric forms, *trans*- and *cis*-isomers, were identified in similar levels for piceid and mainly *trans*- forms for astringin and resveratrol. These compounds were observed in all berry growth stages in different concentrations (Jeandet et al., 1991). They are subject to enzymatic transformations leading to the formation of a pool of different compounds (Chong et al., 2009). The hydroxylation of resveratrol and piceid leads to the formation of piceatannol and its glucoside, astringin (Bavaresco et al., 2002). In addition to these three monomers some other minor monomers were identified such as pterostilbene (Pezet et al., 1994), and isorhapontigenin (Fernández-Marín et al., 2012). The oxidative coupling of resveratrol induces the formation of more complex oligomers including dimers, trimers and tetramers (Takaya et al., 2005). Significant levels of oligomers were reported (Flamini et al., 2013; Rosso et al., 2016). In *Vitis vinifera* cultivars, dimers compounds were identified such as pallidol (Vrhovsek et al., 2012), ϵ -viniferin and δ -viniferin (Flamini et al., 2013).

In addition to these dimers, trimers were identified, like miyabenol C and α -viniferin, and a pool of tetramers including hopeaphenol and isohopeaphenol (Flamini et al., 2013; Flamini et al., 2016; Rosso et al., 2016). Unfortunately, few studies were focused on the quantification of these complex compounds in grape berries (Flamini et al., 2013). In contrast, wild *Vitis* species appear to contain a greater number and diversity of oligomeric stilbenes (He et al., 2009; Jiang et al., 2012).

Table 1. Main stilbenes quantified in berries of different *Vitis vinifera* red grape varieties in mg/kg fresh weight (mean content, standard deviation, minimum and maximum content, number of studies, and number of measures).

Compounds	Mean content (in mg/kg fw)	SD	Min.	Max.	n	N	Ref.
<i>trans</i> -resveratrol	0.67	0.97	0.00	3.56	12	84	(Bavaresco et al., 2002; Brillante et al., 2018; Fernández-Marín et al., 2013; Flamini et al., 2013; Flamini et al., 2016; Guerrero et al., 2010; Kolouchová-Hanzlíková et al., 2004; Rosso et al., 2016; Viñas et al., 2009; Viñas et al., 2011; Vincenzi et al., 2013; Vrhovsek et al., 2012)
<i>cis</i> -resveratrol	0.01	0.07	0.00	0.40	5	38	
Total resveratrol	0.68						
piceatannol	0.12	0.22	0.00	1.35	11	76	
<i>trans</i> -piceid	0.65	1.30	0.00	6.87	7	32	
<i>cis</i> -piceid	0.71	1.61	0.00	6.77	7	32	
Total piceid	1.36						
<i>trans</i> -astringin	0.71	0.58	0.12	1.73	5	7	
<i>cis</i> -astringin	0.12	0.09	0.04	0.29	4	6	
Total astringin	0.83						

Different biotic and abiotic factors affect the stilbene content in grape berries, including the grape varieties or species, stage of ripening, environmental conditions or postharvest treatments (Błaszczyk et al., 2019; Hasan and Bae, 2017). The main factors that can have an impact on stilbene quantities in grapes were described below.

Stage of ripening

The development of grape berries is a dynamic process that involves a complex series of changes divided into three major phases: before veraison, veraison and after veraison. Before veraison, the cell division is rapid and all cells are established within two weeks of flowering, followed by a subsequent sigmoidal increase in berry size (Deluc et al., 2007). At the end of this period, the STS is accumulated and reaches its maximum two weeks before veraison. The stage after veraison is characterized by the initiation of colour development (accumulation of anthocyanins in red grapes) until maturity and the accumulation of stilbenes (Wang et al., 2016). Even if the STS its maximum level two weeks before veraison, the accumulation of stilbenes only start in the second week after veraison and then increases until maturity (Wang et al., 2016).

Grape varieties and species

The accumulation of stilbenes seems to depend on the *Vitis vinifera* grape cultivars. In a comparative study between three different cultivars, Syrah, Tempranillo and Merlot, Guerrero et al. have shown that Syrah had ten times more resveratrol than Tempranillo (Guerrero et al., 2010). This result was confirmed by Gatto et al. that compared 78 different cultivars during three years (Gatto et al., 2008). Firstly, they observed higher stilbene content in red varieties than in white or pink ones. Secondly, Pinots and related cultivars exhibited the highest stilbene levels. Biochemical and transcriptomic analyses have shown the genotype influence on stilbene accumulation in healthy grapevine berries. In the high yielding varieties, glucosylated forms such as piceid were accumulated preferentially, resveratrol being mainly produced after external stimuli such as fungal infection.

Nevertheless, these data have to be substantiated by consistent results because external stimuli, such as light or pathogen infection could have higher effect than the intrinsic differences between cultivars (Hasan and Bae, 2017). As for other secondary metabolites (Acevedo de la Cruz et al., 2012; Hilbert et al., 2015), it is likely that wild

species present higher content and diversity of stilbene than the cultivated one (Jiang et al., 2012). In Southern China grapes (*Vitis chunganensis*), it was reported that resveratrol content twenty times higher than in berries of cultivated grape (He et al., 2009). In addition many oligomers were identified with high contents such as amurensin G (262 mg/kg), amurensin B (242 mg/kg) or r2-viniferin (191 mg/kg). These results are in agreement with the data concerning other parts of the grapevine, such stem or roots. Wild *Vitis* species, that have higher resistance to external stress, seem to contain more stilbenes than cultivated vine (Pawlus et al., 2013b).

External stimuli

Several external stimuli could modulate the stilbene content in grape berries including pathogen infection, elicitor applications, or UVC treatments. The effects of stimuli could increase the stilbene content more than ten times the normal. As stilbenes are well-known phytoalexins (Langcake and Pryce, 1977), the effect of different pathogens on stilbenes accumulation in grape was widely investigated. Infection by *Botrytis cinerea* induce the accumulation of resveratrol mainly in its glucosidic form (Roldán et al., 2003). Similar results were observed for *Plasmopara viticola*, *Uncinula necator* and *Rhizopus stolonifera* (Hasan and Bae, 2017), with accumulation rate ranging between three and twelve times than control. Remarkably, the increase of the resveratrol level in berries due to pathogen infection does not lead to a significant modification of the stilbene content in wines (Jeandet et al., 1995; Roldán et al., 2003). Authors suppose that grape berries with a high *Botrytis* infestation presented lower resveratrol contents due to the oxidation of resveratrol by the laccase enzymes secreted by fungi. In the same manner, chemical compounds such as elicitors are able to induce the production of stilbenes (Krisa et al., 1999). This effect was mainly demonstrated in grape cell cultures using different chemicals such as methyl jasmonate, salicylic acid, glucan, or chitosan (Vuong et al., 2014). The induction could rise up stilbene production to ten times the control. This observation was confirmed in grape berries, where methyl jasmonate induced a significant increase of resveratrol, piceatannol, isorhapontigenin and ϵ -viniferin content up to two times in comparison to the control (Fernández-Marín et al., 2014). Physical processes could also stimulate the production of stilbenes. Pre- and post-harvest treatments by UVC induce an increase of the stilbene content in grape berries (Adrian et al., 2000; Guerrero et al., 2010). The increase rate depends on the variety, the wavelength of the radiation, and the duration of treatment. The

values were mainly ranged between one and twenty times the normal resveratrol concentration (Błaszczuk et al., 2019). In addition to the increase in the amount of resveratrol, levels of other stilbenes rise in the same proportion. This effect was observed on different stilbenes including piceid, piceatannol, ϵ -viniferin, δ -viniferin and hopeaphenol (Guerrero et al., 2016).

Wine stilbenes

Wine is an important dietary source of resveratrol (Kasiotis et al., 2013), in which it can be found at concentrations up to 20 mg/L (Ribeiro De Lima et al., 1999). As for grape berries, the monomeric forms are the most abundant stilbenes in wine, while oligomeric forms were mainly identified in red wines (Guerrero et al., 2020). The main compounds identified in wines were piceid (*cis*- and *trans*-isomers), resveratrol (*cis*- and *trans*-isomers), astringin (*cis*- and *trans*-isomers) and piceatannol (**Tables 2 and 3**). In addition to these compounds several other stilbenes were identified including dimers such as pallidol (Landrault et al., 2002), ϵ -viniferin (Amira-Guebailia et al., 2009; Landrault et al., 2002), δ -viniferin (Moss et al., 2013; Vitrac et al., 2005), ω -viniferin (Guerrero et al., 2020), parthenocissin A (Vitrac et al., 2001), quadrangularin A (Pawlus et al., 2013a); trimers such as miyabenol C (Guerrero et al., 2020), and α -viniferin (Arraki et al., 2017); and tetramers such as hopeaphenol (Guebailia et al., 2006), isohopeaphenol, and r^2 -viniferin (Guerrero et al., 2020). Glucosidic and diglucosidic forms of these compounds were also identified including ϵ -viniferin diglucosides, pallidol 3-O-glucoside, and pallidol diglucosides (Baderschneider and Winterhalter, 2000). In addition, analyses by mass spectrometry indicated the presence of several other oligomers in wines (Moss et al., 2013).

Red wines (**Table 2**) have a higher content in stilbenes than white wines (**Table 3**). Piceid is the main stilbene in wines (means 8.26 and 0.88 mg/L in red and white wines, respectively). Surprisingly, the *cis*-isomer levels are higher in both cases (means 6.20 and 0.68 mg/L in red and white wines, respectively). In red wines, the piceid is followed by resveratrol (mean 2.29 mg/L). The derivatives of piceatannol are present at a concentration of about 1.73 mg/L. Oligomers are minor compounds except isohopeaphenol with a mean value of 1.39 mg/L in red wines (**Table 2**). No oligomers were reported in white wines.

Table 2. Main stilbenes quantified red wines (mean content, standard deviation, minimum and maximum content, number of studies, and number of measures).

Compounds	Mean content (in mg/L)	SD	Min.	Max.	n	N	Ref.
<i>Monomers</i>							
<i>trans-resveratrol</i>	1.41	1,17	0.00	3.75	8	103	
<i>cis-resveratrol</i>	0.88	1.07	0.00	6.08	7	96	(Arraki et al., 2017; Buiarelli et al., 2007; Careri et al., 2004; Guerrero et al., 2020; Kolouchová-Hanzlíková et al., 2004; Lee and Rennaker, 2007; Lukić et al., 2019; Sato et al., 1997; Viñas et al., 2009)
<i>Total resveratrol</i>	2.29						
<i>piceatannol</i>	0.68	0.94	0.00	5.22	3	35	
<i>trans-piceid</i>	2.06	2.19	0.00	9.31	6	82	
<i>cis-piceid</i>	6.20	8.09	0.00	38.47	6	75	
<i>Total piceid</i>	8.26						
<i>trans-astringin</i>	0.52	0.61	0.00	3.00	3	32	
<i>cis-astringin</i>	0.13	0.32	0.00	1.59	2	28	
<i>Total astringin</i>	0.65						
<i>Dimers</i>							
<i>trans-ε-viniferin</i>	0.06	0.21	0.00	0.81	2	15	
<i>trans-ω-viniferin</i>	0.03	0.10	0.00	0.30	1	10	
<i>Others</i>							
miyabenol C	0.14	0.45	0.00	1.41	1	10	
hopeaphenol	0.08	0.15	0.00	0.49	2	15	

Isohopeaphenol	1.39	1.80	0.00	7.47	3	19
R2-viniferin	0.42	1.05	0.00	3.28	1	10

Table 3. Main stilbenes quantified white wines (mean content, standard deviation, minimum and maximum content, number of studies, and number of measures).

Compounds	Mean content (in mg/L)	SD	Min.	Max.	n	N	Ref.
<i>trans</i> -resveratrol	0.08	0.20	0.00	1.16	6	55	(Arraki et al., 2017; Buiarelli et al., 2007; Guerrero et al., 2020; Lee and Rennaker, 2007; Lukić et al., 2019; Sato et al., 1997; Viñas et al., 2009)
<i>cis</i> -resveratrol	0.04	0.13	0.00	0.76	5	55	
Total resveratrol	<i>0.12</i>						
piceatannol	0.04	0.16	0.00	0.59	3	14	
<i>trans</i> -piceid	0.20	0.39	0.00	1.91	6	61	
<i>cis</i> -piceid	0.68	2.23	0.00	16.26	6	61	
Total piceid	<i>0.88</i>						
<i>trans</i> -astringin	0.05	0.16	0.00	0.72	3	20	
<i>cis</i> -astringin	0.11	0.35	0.00	1.32	2	14	
Total astringin	<i>0.16</i>						

Several parameters are able to modulate the stilbene content in wine including mainly the extraction from grape, but also yeast strains, and enological practices.

Duration of maceration

Red wines contain higher stilbene levels than white wines that are obtained from a limited maceration with the pomaces (**Tables 2 and 3**). In fact, the stilbene content in wines is strongly modulated by the duration of the maceration and the solubility of these compounds in alcohol (Kostadinović et al., 2012; Mattivi et al., 1995). It has been shown that wines obtained after 6 days of maceration had the highest concentration of resveratrol and piceid than wine obtained after 3 days (Kostadinović et al., 2012), reaching to the maximal content (Poussier et al., 2003). Due to their polarity, monomeric glucosides are extracted before their aglycone forms (Mattivi et al., 1995). While oligomers such as δ -viniferin are observed in wines after few days of maceration (Poussier et al., 2003). After a long time of maceration the concentration of stilbenes decrease mainly that of glucosides such as *cis*- and *trans*-piceid (Poussier et al., 2003). The decrease of piceid could be attributed to the β -glucosidase activity of the different yeasts (Jeandet et al., 1994). In addition to this phenomenon, the *cis/trans* isomerization of stilbenes could explain the formation of some of these compounds such as *cis*-resveratrol. The levels of this compound are similar to those of *trans*-resveratrol in red wines (**Table 2**) and significantly lower in grapes (**Table 1**). Finally, even if few studies about resveratrol degradation during maceration were conducted, yeasts seem to be able to induce a degradation of resveratrol during wine fermentation (Vacca et al., 1997).

Yeast activities

As previously mentioned, yeast activities influence the stilbene content in wines. A comparison between French and Macedonian yeasts demonstrated a variation of resveratrol content up to four times depending on the yeast and maceration times (Kostadinović et al., 2012). The β -glucosidase activities of yeasts induce the hydrolyze of glucosides which leads to the formation of non glucosylated stilbenes (Mattivi et al., 1995). Thus, the selection of exogenous yeasts could significantly affect the stilbene content in wines. The use of specific yeasts enriched in β -glucosidase could positively

increase the level of resveratrol in wines (González-Candelas et al., 2000). Nevertheless more studies are needed to better understand the impact of yeast activities on stilbene content in wines.

Winemaking processes and wine ageing

Few studies were focused on the impact of winemaking processes on the stilbene content in wines. Grapes exposure to UVC radiations before winemaking induces an increase of stilbene production in wines (Cantos et al., 2003; Threlfall et al., 1999). This positive effect is observed on monomeric stilbenes such as resveratrol and piceatannol whereas other stilbenes such as ϵ -viniferin are not affected (Fernández-Marín et al., 2014). On the contrary, the use of some fining agents, such as polyvinylpolypyrrolidone (PVPP), could reduce the stilbene content in wines (Threlfall et al., 1999; Vrhovsek et al., 1997).

As for winemaking processes few studies were focusing on the stilbene stability during wine ageing. Stilbenes are known to be relatively stable compounds (Bavaresco et al., 2012; Gaudette and Pickering, 2011). Nevertheless, even if the total stilbene content is not affected, the ratio between resveratrol and piceid isomers could be impacted during wine ageing (Favre et al., 2020; Sun et al., 2006). In addition some specific ageing processes such as those used in Sherry wines could reduce the resveratrol content (Roldán et al., 2010). Finally, heat could induce oxidative coupling between monomeric stilbenes inducing the formation of oligomers in wines (El Khawand et al., 2020).

Analytical methods for stilbenes analysis in grapes and wines

The relatively low concentration of stilbenes, their structural diversity, and the complexity of grape and wine matrices restrain their identification and quantification. Today, the studies are mainly focused on the monomeric stilbenes. Several analytical procedures were developed in order to determine the stilbene content in grapevines and wines from monomers to complex oligomers. These strategies often require multi-steps sample preparation (Baderschneider and Winterhalter, 2000; Liu et al., 2013; Romero-Pérez et al., 2001).

Concerning grapevines, the first step consists of the extraction of stilbenes from the

raw material. Several extraction procedures were applied including classical solvent extraction from fresh, frozen, dried or lyophilized berries (Romero-Pérez et al., 2001), or more recently ultra sonication-assisted extraction (Cho et al., 2006). The classical solvent extraction remains the most applied technique the main used methodology using acetone, chloroform, ethanol, ethyl acetate, methanol, and some mixed solvents. Methanol, pure or mixed, is the most widely used solvent for stilbene extraction from grapevine berries. Several studies were focused on optimizing the extraction conditions from grapevine berries (Liu et al., 2013; Romero-Pérez et al., 2001; Sun et al., 2006). The main parameters analyzed were the solvent, the extraction time, the temperature, and the ratio solid to solvent. Based on Liu et al. (2013) studies (Liu et al., 2013), the best extraction solutions were: methanol or methanol/ethyl acetate (1:1 (v/v)) with a ratio solid to solvent of 1 g/10 mL at 25°C for 24 h. In addition, in order to avoid *cis/trans* isomerization, extraction should be carried out in the dark (Careri et al., 2003). Interestingly, innovative extraction techniques such as ultrasonication-assisted extraction seem to be able to increase the stilbene extraction rate while reducing time and solvent consumption (Cho et al., 2006).

As for grape berries, several methods were proposed to extract stilbenes in wines, from direct analyses (Lamuela-Raventós et al., 1995) to more complex procedures using solid phase extraction (Mattivi et al., 1995), stir bar sorptive extraction (Viñas et al., 2008), microextraction by packed sorbents (Gonçalves and Câmara, 2011), liquid–liquid extraction (Rabesiaka et al., 2011), dispersive liquid–liquid microextraction (Rodríguez-Cabo et al., 2012), semi preparative liquid chromatography (Amira-Guebailia et al., 2009), or counter current chromatography (Fernandez-Marin et al., 2012). The main drawback of these methods is that they are mainly focused on resveratrol and piceid. In the research conducted by Baderschneider and Winterhalter a combination of solid phase extraction and counter current chromatography were used to identify new stilbene skeletons in wines (Baderschneider and Winterhalter, 2000).

Several methods were used for stilbene analysis in grape berries and wines including capillary electrophoresis (CE), gas chromatography (GC), high performance liquid chromatography (HPLC) and ultra-high performance liquid chromatography (UHPLC).

Capillary electrophoresis

The capillary electrophoresis is appropriate for the separation of small molecules and could separate some optical isomeric forms. This method allows separating positional isomers as well as optical isomers. This method has different advantages such as fast and accurate analysis, low amount and consumption (Coelho et al., 2016). This technique was successfully applied for resveratrol analysis in wines (Gu et al., 2000), and to discriminate *cis*- and *trans*-resveratrol (Paulo et al., 2011). Nevertheless, this method was used to identify a limited number of stilbenes. In addition, due to its low sensitivity, concentrating the wine sample is recommended using solid phase or liquid–liquid extractions before proceeding to capillary electrophoresis (Gu et al., 2000; Spanilá et al., 2005).

Gas chromatography

Gas chromatography was successfully used for resveratrol quantification in wines (Barbanti et al., 1996), and grapes (Viñas et al., 2009). The use of GC for stilbene analysis involves the derivatization of the compound using derivatizing reagents, such as bis(trimethylsilyl)trifluoroacetamide (BSTFA) (Luan et al., 2000), or acetic anhydride (Montes et al., 2010), in order to increase their volatility. Silylation is the most common derivatization reaction used in combination with solid phase or liquid-liquid extraction pretreatments for grape and wine analyses (Cai et al., 2009; Viñas et al., 2009). In addition, GC analyses were coupled with mass spectrometry detector (GC–MS) in order to increase the accuracy and sensibility of the detection (Rodríguez-Cabo et al., 2016). These methods provide limits of detection close to ng/L in wines. However, GC analyses have different drawbacks such as isomerization, degradation of analytes.

Liquid Chromatography

High-performance liquid chromatography (HPLC) is considered among the most commonly applied method for stilbenes analysis in grapes (Błaszczyk et al., 2019) and wines (Fabjanowicz et al., 2018), using different detection systems such as UV-visible or diode array detectors (Mattivi et al., 1995; Sun et al., 2006), or fluorescence detectors in order to increase sensitivity and specificity (Vitrac et al., 2002). Nowadays,

HPLC combined with mass spectrometry (HPLC-MS) has become the most used technique for the determination of phenolic compounds in general and stilbenes in particular (Pugajeva et al., 2018). This method allows the identification of all stilbenes (free and conjugated) without any derivatization or hydrolysis of samples. Different mass detectors were used including triple quadrupole (QqQ) and quadrupole time-of-flight (QTOF). Information on liquid chromatography methods developed for stilbenes identification and quantification in grape berries and wines are summarized in [Table 4](#).

Table 4. Fragmentation patterns and tentative assignments of stilbenes in red wines (Moss et al., 2013).

Compounds	RT (Min)	Formula	Calculated	Experimental	Mass error (ppm)	CE (eV)	MS/MS product ions
<i>trans</i> -resveratrol	20.005	C ₁₄ H ₁₁ O ₃	227.0714	227.0725	4.84	22.5	185; 143
<i>cis</i> -resveratrol	21.96	C ₁₄ H ₁₁ O ₃	227.0714	227.0717	1.32	22.5	185; 143
piceatannol	14.242	C ₁₄ H ₁₁ O ₄	243.0663	243.0659	-1.65	25	201; 159
<i>trans</i> -piceid	11.185	C ₂₀ H ₂₁ O ₈	389.1242	389.1235	-1.8	20	227
<i>cis</i> -piceid	13.561	C ₂₀ H ₂₁ O ₈	389.1242	389.1263	5.4	20	227
astringin	8.209	C ₂₀ H ₂₁ O ₉	405.1191	405.1206	3.7	15	243; 201; 159
pallidol	21.459	C ₂₈ H ₂₁ O ₆	453.1344	453.1347	0.66	20	359; 265
parthenocissin A	23.959	C ₈ H ₂₁ O ₆	453.1344	453.1344	-4.19	20	359; 289
ampelopsin D	26.765	C ₂₈ H ₂₁ O ₆	453.1344	453.1363	4.19	20	359; 289
<i>cis</i> -ε-viniferin	31.448	C ₂₈ H ₂₁ O ₆	453.1344	453.1345	0.22	20	435; 411; 369; 359; 347; 333; 225
<i>trans</i> -ε-viniferin	33.833	C ₂₈ H ₂₁ O ₆	453.1344	453.1376	7.06	20	435; 411; 369; 359; 347; 333; 225
<i>cis</i> -ω-viniferin	32.202	C ₂₈ H ₂₁ O ₆	453.1332	453.1344	-2.65	20	435; 411; 369; 359; 347; 333; 225
<i>trans</i> -ω-viniferin	34.34	C ₂₈ H ₂₁ O ₆	453.1344	453.1357	2.87	20	435; 411; 369; 359; 347; 333; 225
<i>cis</i> -δ-viniferin	39.705	C ₂₈ H ₂₁ O ₆	453.1344	453.1329	-3.31	25	453; 411; 369; 359; 333
<i>trans</i> -δ-viniferin	38.789	C ₂₈ H ₂₁ O ₆	453.1344	453.1356	1.77	25	435; 411; 369; 359; 333
<i>trans</i> -scirpusin A	27.93	C ₂₈ H ₂₁ O ₇	469.1293	469.1307	2.98	25	451; 427; 385; 375; 359; 347; 333; 241
restrisol A	11.303	C ₂₈ H ₂₃ O ₇	471.1449	471.1447	-0.42		377; 349; 255; 121
parthenostilbenin A	19.64	C ₂₉ H ₂₅ O ₇	485.1606	485.1635	5.98	15	453; 391; 359; 289; 255; 187
parthonostilbenin B	21.088	C ₂₉ H ₂₅ O ₇	485.1606	485.1594	-2.47	15	453; 391; 359; 289; 255; 187

ϵ -viniferin glucoside	25.202	C ₃₄ H ₃₁ O ₁₁	615.1872	615.1878	0.98	20	453; 411; 359; 347
ampelopsin C	30.044	C ₄₂ H ₃₂ O ₉	679.1974	679.1981	1.03	30	585; 573; 491; 479; 385
<i>trans</i> -miyabenol C	35.372	C ₄₂ H ₃₂ O ₉	679.1974	679.1978	0.59	30	661; 637; 585; 573; 555; 451; 479; 357; 345
<i>cis</i> -miyabenol C	36.322	C ₄₂ H ₃₂ O ₉	679.1974	679.1984	1.47	30	661; 637; 585; 573; 555; 479; 451; 357; 345
hopeaphenol	31.695	C ₅₆ H ₄₁ O ₁₂	905.2604	905.2573	-3.42	35	811; 717; 611; 451; 359; 265

In recent years, UHPLC coupled with mass spectrometry (UHPLC-MS) has been widely used as an alternative for other methods due to its higher sensitivity, accuracy, rapidity, and low solvent consumption for grape berry (Flamini et al., 2013), and wine analyses (Guerrero et al., 2020; Moss et al., 2013). For example, based on a targeted metabolomics approach using UHPLC-QTOF mass spectrometer, Flamini et al. have identified and quantified eighteen stilbenes in grape berries including oligomers. In wines, an UHPLC-QqQ-MS method was designed to identify and quantify fifteen stilbenes in white and red wines with limit of detection ranging between 4 and 28 µg/L (Guerrero et al., 2020). Similarly, using a UHPLC-QTOF mass spectrometer, forty-one stilbenes were identified in red wines, including six monomers, twenty-three dimers, eight trimers and four tetramers (**Table 5**).

Due to the complexity and the diversity of stilbene structures, pure compounds are needed as standard to obtain accurate results. Unfortunately, since pure samples of complex stilbene oligomers are often unavailable, results are expressed in resveratrol or piceid equivalent (Flamini et al., 2013). This approach could lead to severe underestimation of the oligomeric stilbene content (Biais et al., 2017). For example, the quantification of ϵ -viniferin using resveratrol by a HPLC-DAD method underestimated the concentration ϵ -viniferin, R-viniferin and isohopeaphenol by a factors upper than two, five and ten, respectively.

Table 5. Information on liquid chromatography methods for the identification and quantification of stilbenes in grape berries and wines.

Compounds	Sample preparation	Analytical methods	LOD	LOQ	References
1,2	<p>Wine: direct injection</p> <p>Grape skin: extraction ethanol (80%)</p> <p>Standard used: <i>trans</i>- and <i>cis</i>- resveratrol</p>	HPLC- UV	3-15 µg/L	-	(Kolouchová-Hanzlíková et al., 2004)
1, 7, 9, 15, 24	<p>Wine: extraction ethyl acetate</p> <p>Grape berries: water/acetonitrile (1:1, v/v)</p> <p>Standard used: pure compounds</p>	HPLC-UV	wine: 5-8 ng	-	(Landrault et al., 2002)
1, 7, 8	<p>Grape skin: extraction with various solvent</p> <p>Standard used: pure compounds</p>	<p>UPLC-MS/MS (identification)</p> <p>HPLC-DAD (quantification)</p>	-	-	(Sun et al., 2006)
1	<p>Wine: direct injection</p> <p>Grape skin and pomace: extraction methanol/ethanol (4:1, v/v)</p> <p>Standard used: <i>trans</i>-resveratrol</p>	<p>HPLC-UV</p> <p>HPLC-ESI-MS/MS</p>	10 µg/L	16 µg/L	(Careri et al., 2004)
1, 3, 15, 20	<p>Grape skin: extraction methanol</p> <p>Standard used: <i>trans</i>-resveratrol</p>	<p>UPLC-MS/MS (identification)</p> <p>HPLC-DAD (quantification)</p>	0.01 mg/kg	0.04 mg/kg	(Guerrero et al., 2010)
1, 2, 9, 14, 15, 16, 17, 24, 27, 28, 29, 31	<p>Wine: direct injection</p> <p>Grape berries: extraction water/methanol/chloroform (1:2:2, v/v/v)</p>	UPLC-QqQ-MS/MS	-	8-400 pg	(Vrhovsek et al., 2012)

	Standard used: pure compounds				
1, 2, 3, 7, 8, 9, 10, 12, 15, 17, 28, 30, 31, 32, 33	Wine: direct injection Standard used: pure compounds	UPLC-QqQ-MS/MS	5-28 µg/L	12-84 µg/L	(Guerrero et al., 2020)
1, 3, 7, 9	Wine: solid-phase extraction (SPE) protocol Standard used: <i>trans</i> -resveratrol, <i>trans</i> -piceid, <i>trans</i> -piceatannol	UPLC-QTOF-MS/MS (identification) UPLC-QqQ-MS/MS (quantification)	48-50 µg/L	160-167 µg/L	(Buiarelli et al., 2007)
1, 3, 7, 8, 9, 10, 11, 14, 15, 17, 19, 24, 27, 28, 29, 30, 31	Grape berries: extraction methanol Standard used: <i>trans</i> -resveratrol, <i>trans</i> -piceid, ε-viniferin	UPLC-QTOF-MS/MS	-	-	(Flamini et al., 2013; Flamini et al., 2016; Rosso et al., 2016)
1, 2, 3, 7, 8, 9, 11, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 28, 29, 30, 31	Wine: extraction diethyl ether	UPLC-QTOF-MS/MS	-	-	(Moss et al., 2013)

Stilbenes, *in vitro* and *in vivo* anti-obesity effects and molecular mechanisms

Anti-obesity *in vitro* effects of stilbenes

As was noted in the Introduction section, stilbenes might act as anti-obesity agents by regulating different fat metabolism pathways such as adipogenesis, lipogenesis, lipolysis and thermogenesis (Chou et al., 2018; Fernández-Quintela et al., 2017).

Adipogenesis is defined as the differentiation process of preadipocytes to fully mature to adipocytes. This process can be divided in different stages: growth phase and growth arrest, clonal expansion, gene expression of lipogenic proteins inducing triglyceride (TG) accumulation and differentiation and cell death (apoptosis) (Esteve Ràfols, 2014). Some transcription factors like the peroxisome proliferator-activated receptor gamma (PPAR γ), the sterol regulatory element binding protein 1c (SREBP-1c) and CCAAT-enhancer binding proteins (C/EBPs) are key elements in production of the fully mature adipocytes (Rosen et al., 2002). Moreover, others proteins such as glucose transporter 4 (GLUT4), adipocyte protein 2 (aP2) or lipoprotein lipase (LPL) are also involved in this process (MacDougald and Mandrup, 2002).

Concerning the anti-adipogenic effects of stilbenes, the most investigated molecule was resveratrol. Several papers demonstrated that resveratrol is able to inhibit preadipocyte and adipocyte differentiation through the decrease of gene and protein expressions of PPAR γ , C/EBP α and C/EBP β at concentrations ranging between 0.03-400 μ M in mouse, bovine and human cells (**Table 6**). In addition to suppressing the expression of several key lipogenic genes, resveratrol can interfere by diminishing preadipocyte proliferation and inhibiting the clonal expansion stage or the cell cycle entry to G2/M phase. Actually, a decrease of the expression of cell cycle genes, such as cyclin D1 and A2, cyclin-dependent kinase 2 and 4, and DNA-damage inducible transcript 3 (Ddit3, also known as Chop-10), was observed (Kwon et al., 2012b; Mitterberger and Zwerschke, 2013; Santos et al., 2014). Furthermore, resveratrol interacted with the insulin receptor (IR) in 3T3-L1 preadipocyte cells and inhibited the insulin signaling pathway in the early phase of adipogenesis (Kwon et al., 2012b).

More recently, a very interesting work of Eseberri et al. showed that some resveratrol metabolites, and specifically resveratrol 3'- and 4'-glucuronide (at 25 μ M concentration), were able to regulate and inhibit the expression of C/EBP β and

Krüppel-like factor (KLF9) that mediates both the early and late stages of the differentiation program (Eseberri et al., 2017).

Some of these studies, also showed that resveratrol decrease or attenuates the production of other key adipogenic proteins such as fatty acid binding protein 4 (FABP4) that regulates adipogenesis by downregulating PPAR γ (Garin-Shkolnik et al., 2014). Santos et al. also reported an important modulation role of resveratrol on the gene expression of Adipogenic, Bone morphogenetic protein 2 (Bmp2), fatty acid synthase (FAS), fibroblast growth factor 10 (Fgf10) and leptin (Santos et al., 2014). Likewise, this lipid-lowering effect has been associated and depends on the sirtuin 1-AMP-activated protein kinase-Forkhead box protein O1 (SIRT1-AMPK-FOXO1) pathway (Liu et al., 2018). It is well know that SIRT 1 is responsible of fat mobilization in mature adipocytes and its activation give rise to the inhibition of PPAR γ expression (Picard et al., 2004). Additionally, AMPK pathway plays an important role on the control of body fat stress. In fact, some studies have demonstrated that hormones, such as leptin and adiponectin, adrenergic agonists, and metformin, activate AMPK in adipocytes (Rossmeisl et al., 2004). AMPK negatively regulates white adipogenesis, specifically blocking the clonal expansion of preadipocytes by attenuating adipocyte differentiation (Kang et al., 2005). In addition, AMPK activation in the early phase of differentiation inhibits PPAR γ and C/EBP α expression as well as late adipogenic markers such as FAS and acetylCoA carboxylase (ACC). For these reasons, it is well accepted that AMPK activity is inversely related to white adipogenesis.

Moreover, resveratrol can induce the cell death in both mice 3T3-L1 cells (Rayalam et al., 2008), and human pre-adipocytes (Liu et al., 2018). In fact, a modulation of the expression of caspase-3, and the pro-apoptotic protein Bax was demonstrated after resveratrol treatment that also implicated the SIRT1-AMPK-FOXO1 pathway (Liu et al., 2018).

Concerning other stilbenes, r- and r2-viniferin, stilbene tetramers that were recently identified in wine (Guerrero et al., 2020), are also thought to be able to inhibit adipocytes differentiation and reduce lipid accumulation in 3T3-L1 cells by decreasing the expression of PPAR γ , C/EBP α and FABP4 genes (Tie et al., 2018). In accordance with these results, r2-viniferin suppressed the adipogenic process by blocking the cell cycle at the G1-S phase through p21- (CDK inhibitor) and Rb-dependent suppression

of transcription in 3T3-L1 cells (Kim et al., 2008). Long-term treatment at low concentrations (5-10 μ M) by pterostilbene, a methylated derivative of resveratrol, inhibited adipocyte differentiation in 3T3-L1 preadipocytes and 3T3-F442A cells (Gomez-Zorita et al., 2017; Hsu et al., 2012). This compound induced heme-oxygenase 1 (HO-1) expression which acts as a regulator of Chop10, suppressing in consequence the initiation of mitotic clonal expansion (Seo et al., 2017). Piceatannol acts in the early phase of adipogenesis delaying the cell cycle entry into G2/M phase at 24 h after initiation of adipogenesis and suppressing the mitotic clonal and the activation of the insulin-signaling pathways (Kwon et al., 2012a). Furthermore, ϵ -viniferin, a resveratrol dimer, showed anti-adipogenic effects by downregulating PPAR γ mRNA levels at 50 μ M concentration (Ohara et al., 2015).

Additionally, the combination of stilbenes with other polyphenols may present synergistic or additional effects. Some studies proved that the combination of resveratrol with genistein, quercetin or epigallocatechin gallate enhanced the resveratrol efficiency by inhibiting adipogenesis and decreasing the lipid accumulation and TG content in 3T3-L1 and human primary adipocytes cells (**Table 6**). More specifically, the treatment with these compounds (30 μ M) decreased the protein expression of PPAR γ , C/EBP α , FABP4 and perilipin (Ahmed et al., 2017). Additionally, an increase of the apoptotic process has also been observed in early- and mid-phase maturing and lipid-filled mature human primary adipocytes after resveratrol, genistein and quercetin treatment (Park et al., 2008).

Lipogenesis process involves *de novo* fatty acid and TG synthesis from glucose metabolism products (Wang et al., 2004). In human body this pathway is active in liver and adipose tissue. Once glucose is incorporated into the cells, it undergoes a series of biochemical transformations (glycolysis) to produce acetyl-CoA. This last compound is transformed in malonyl CoA by acetyl-CoA carboxylase (ACC) which is a substrate for fatty acid synthase (FAS) to synthesize fatty acids. Two other enzymes, malic enzyme (ME) and glucose-6-phosphate dehydrogenase (G6PDH) supply NADPH to fatty acid synthesis reactions. SREBP-1c has also an important role as a transcription factor by regulating the expression of ACC and FAS (Vázquez-Vela et al., 2008).

Table 6. *In vitro* effects of resveratrol, other stilbenes and combination of stilbenes and other bioactives on adipogenesis, apoptosis, lipogenesis, lipolysis and thermogenesis.

ADIPOGENESIS & APOPTOSIS			
Resveratrol and metabolites	Cell model	Effect	Reference
100, 200, 400 μ M	Bovine intramuscular adipocytes	<p>↓ PPARγ, FAS</p> <p>↑Caspase-3, Bax</p> <p>↑SIRT1, AMPKα, FOXO1</p>	(Liu et al., 2018)
0.03-100 μ M	3T3-L1 cells	↓PPAR γ , perilipin	(Chang et al., 2016)
1, 10, 50, 100 μ M	3T3-L1 cells	<p>↓ PPARγ, C/EBPα, FABP4</p> <p>↓adipocyte differentiation</p>	(Hu et al., 2015)
50-300 μ g/mL	3T3-L1 cells	<p>↓ PPARγ,C/EBPα, CEBPβ, FAS, FGF10, leptin, LPL, adipogenin, BMP2, cyclin D1, cyclin-dependent kinase 4, FGF2, KLF2, KLF15, DDIT3</p> <p>↑FOXO1, SIRT1, SIRT2</p>	(Santos et al., 2014)
100 μ M	3T3-L1 cells	<p>↓Cell cycle entry (↓AKT, MAPK, cyclin D1).</p> <p>↓Clonal expansion (cyclin A2)</p>	(Mitterberger and Zwerschke, 2013)
25-50 μ M	3T3-L1 cells	<p>↓Clonal expansion</p> <p>↓Cell cycle entry of preadipocytes to G2/M phase (cyclin A and cyclin dependent kinase 2).</p> <p>↓IR activity (<u>through a direct physical interaction between resveratrol and IR</u>)</p>	(Kwon et al., 2012b)
200 and 400 μ g/mL (grape skin extracts)	3T3-L1 cells	<p>↓PPARγ, C/EBPα, FAS, aP2, SCD-1, LPL</p> <p>↓Lipid accumulation and glycerol-3-phosphate dehydrogenase activity</p>	(Zhang et al., 2012)
100-200 μ M	3T3-L1 cells	<p>↓ PPARγ, C/EBPα, SREBP-1c, FAS, HSL, LPL</p> <p>↓Apoptosis</p>	(Rayalam et al., 2008)

5-100 µmol/L	SGBS cells	↓Preadipocyte proliferation and adipogenic differentiation ↑SIRT-1 ↓IL-6, IL-8	(Fischer-Posovszky et al., 2010)
1 µM	Human visceral adipocytes	↓ PPARγ ↑ SIRT1, FOXO1 and adiponectin	(Costa et al., 2011)
resveratrol-3-O-sulfate (R3S)	3T3-L1 cells	↓C/EBPβ (R3G, R4G) ↓CREB1 (R3S) ↓KLF5 (R3S, R4G), SREBP-1c, LXRα (R3S)	(Eseberri et al., 2017)
resveratrol-3'-O-glucuro (R3G)			
resveratrol-4'-O-glucuronide (R4G)			
25 µM			

Other stilbenes	Cell model	Effect	Reference
Pterostilbene 5-10 μ M	3T3-L1 cells	↓Cell cycle entry at the G2/M phase	(Hsu et al., 2012)
		↓Fat droplet formation and triacylglycerol accumulation, GPDH	
		↓PPAR γ and C/EBP α	
		↑Adiponectin	
Pterostilbene 0-6 μ M	3T3-L1 cells	↓C/EBP α , C/EBP β , PPAR γ , α 2, CHOP10	(Seo et al., 2017)
		↓lipid accumulation	
		↑HO-1	
Vitisin A 10 μ M	3T3-L1 cells	↓Cell cycle at the G1-S phase	(Kim et al., 2008)
		↑p21 expression (CDK inhibitor)	
		↓Rb phosphorylation	
Vitisin A, Vitisin B and <i>cis</i> -vitisin A 10 μ M	3T3-L1 cells	↓PPAR γ	(Tie et al., 2018)
		↓Adypocytes differentiation	
		↓Lipid accumulation	
ϵ -viniferin 25-50 μ M	3T3-L1 cells	↓PPAR γ	(Ohara et al., 2015)
		↓Lipid accumulation	
		↓MCP-1	
Combinations stilbenes or other polyphenols and bioactives			
Resveratrol (25 μ M) + Quercetin (100 μ M)	3T3-L1 cells	↓ PPAR γ , C/EBP α	(Yang et al., 2008)
		↑Cytochrome C from mitochondria to cytosol	
		↓ERK 1/2	

Resveratrol + Genistein (50 and 100 mmol/L)	3T3-L1 cells	<p>↓ PPARγ, CCAAT</p> <p>↑ JNK phosphorylation</p> <p>↓ Lipid accumulation</p>	(Rayalam et al., 2007)
Resveratrol + CLA (10 and 100 μ M)	3T3-L1 cells	<p>↓ TG content</p> <p>↑ ATGL</p> <p>No change in FAS and HSL</p>	(Lasa et al., 2011)
Resveratrol (12.5 and 100 μ M) Genistein (6.25 and 50 μ M) + Quercetin (12.5 and 100 μ M)	Primary human adipocytes (HAs) 3T3-L1 cells (MAs)	<p>↓ Lipid accumulation and glycerol 3-phosphate dehydrogenase activit</p> <p>↑ Apoptosis</p> <p>↓ Lipid accumulation</p>	(Park et al., 2008)
Resveratrol + Genistein + EGCG (30 μ M)	3T3-L1 cells Human primary preadipocytes cells	<p>↓ Preadipocyte differentiation</p> <p>↓ PPAR-γ, C/EBPα</p> <p>↓ FABP4, perilipin</p>	(Ahmed et al., 2017)

LIPOGENESIS (adipose and hepatic)

Stilbene and dose	Model	Effect	Reference
Resveratrol 25-100 µM	3T3-L1 cells Human SGBS	↓Lipogenesis (adipose) ↓ACC ↓Insulin-induced (via AKT and AMPK)	(Li et al., 2016)
Resveratrol 10-80 µmol/L	3T3-L1s cells	↓Lipogenesis (adipose) ↓SREBP-1c, PPAR γ ↑p-AMPK	(Chen et al., 2011)
Resveratrol 5-20 µM	3T3-L1 Adipocytes derived from primary mouse embryonic fibroblasts (MEF)	↓Lipogenesis (adipose) ↓PPAR γ , GLUT4 ↓TG content	(Mercader et al., 2011)
Resveratrol 0-250 µM	Rat adipocytes (from epididymal tissue)	↓Lipogenesis (adipose) ↓Glucose conversion to lipids	(Szkudelska et al., 2009)
Resveratrol 0-25 µg/mL	3T3-L1 cells	↓Lipogenesis (adipose) ↓FAS	(Liang et al., 2013)
Resveratrol + phenelzine (1 µM-1 mM)	Adipocytes isolated from epididymal, retroperitoneal, perirenal and inguinal adipose tissues	↓Incorporation of glucose into lipids ↓Hexose uptake Non synergistic effects	(Carpéné et al., 2014)
Pterostilbene 5-10 µM	3T3-L1 cells	↓Lipogenesis (adipose) ↓PPAR γ ↓FAS ↓GPDH	(Hsu et al., 2012)

Resveratrol 0-30 μ M	Primary hepatocyte HepG2	<p>↓Lipogenesis (hepatic)</p> <p>↓SREBP-1c</p> <p>↓LXRα</p> <p>↑SESN2 expression</p>	(Jin et al., 2013)
Resveratrol 1-100 μ M	Hepatocytes from male Wistar rats	<p>↓Lipogenesis (hepatic)</p> <p>↓Total fatty acid synthesis</p> <p>↓ACC</p>	(Gnoni and Paglialonga, 2009)
Resveratrol + 18 resveratrol synthesized derivatives 10-50 μ M	H4IIEC3 cells HepG2 cells	<p>↓Lipogenesis (hepatic)</p> <p>↓SREBP-1c, FASN, SCD-1</p> <p>↓Lipid content</p>	(Choi et al., 2014)
Oxyresveratrol 30 μ M	HepG2 cells	<p>↓Lipogenesis (hepatic)</p> <p>↓SREBP-1c, FAS, SCD-1</p> <p>↓LRX agonists</p>	(Lee et al., 2018)

LIPOLYSIS

Stilbene and dose	Model	Effect	Reference
Resveratrol 100, 200, 400 μ M	Bovine intramuscular adipocytes	<p>↓PPARγ</p> <p>↑HSL, ATGL</p>	(Liu et al., 2018)
Resveratrol 0.03-10 μ M	3T3-L1 cells	<p>↓PPARγ</p> <p>↑glycerol release</p> <p>↓TNFα-induced lipolysis</p>	(Chang et al., 2016)
Resveratrol + CLA 10 and 100 μ M	3T3-L1 cells	<p>↑ATGL</p> <p>↓TG content</p>	(Lasa et al., 2011)
Resveratrol	3T3-L1 cells	↑FFA release	(Lasa et al., 2012)

100 μ M	SGBS cells	\uparrow ATGL	
Resveratrol 50, 75 and 100 μ M	SGBS cells	\downarrow TG content \uparrow Glycerol release	(Rosenow et al., 2012)
Resveratrol 1mM	3T3-F442A cells	\uparrow Glycerol release	(Gomez-Zorita et al., 2017)
Resveratrol 10-100 μ M	Rat adipocytes (epididymal tissue)	\uparrow Glycerol release \uparrow Epinephrine-induced lipolysis	(Szkudelska et al., 2009)
Pterostilbene 100 nM and 10 μ M	3T3-F442A cells	\uparrow Glycerol release induced by isoprenaline	(Gomez-Zorita et al., 2017)
Resveratrol + genistein (25 mmol/L)	3T3-L1 cells	\downarrow PPAR γ \uparrow Glycerol release	(Rayalam et al., 2007)
Resveratrol 20 μ M	3T3-L1 cells Primary MEF	\uparrow β -oxidation \uparrow CPT-1 \downarrow rip 140	(Mercader et al., 2011)
Oxyresveratrol 30 μ M	HepG2 cells	\uparrow β -oxidation \uparrow CPT-1	(Lee et al., 2018)

THERMOGENESIS/MITOCHONDRIAL BIOGENESIS

Stilbene and dose	Model	Effect	Reference
Resveratrol 10-40 μ M	Vascular cells isolated from iBAT	<p>↑ PRDM16</p> <p>↑ UCP-1 and cytochrome C, PGC1α, PDH</p> <p>↑AMPKα</p>	(Wang et al., 2017)
Resveratrol 25-100 μ M	3T3-L1 cells Human SGBS	<p>↑ ATAD3</p>	(Li et al., 2016)
Resveratrol 5-20 μ M	3T3-L1 cells Adipocytes derived from primary mouse embryonic fibroblasts (MEF)	<p>↑Mitochondrial DNA content</p> <p>↑Subunit II of cytochrome oxidase</p> <p>↑UCP-1</p> <p>↓RBP4, resistin</p>	(Mercader et al., 2011)
Resveratrol 10 μ M	primary stromal vascular cells separated from iWAT	<p>↑ UCP-1, PRDM16, Cell death-inducing DFFA-like effector A (Cidea), elongation of very long chain fatty acids protein 3 (Elovl3), PGC1α, cytochrome C and PDH</p> <p>↑AMPKα1 phosphorylation</p>	(Wang et al., 2015)
Resveratrol 100-200 μ M	3T3-L1 cells	↑ SIRT3, UCP-1, Mfn2	(Rayalam et al., 2008)
Resveratrol 50-300 μ g/mL	3T3-L1 cells	↑ UCP-1	(Santos et al., 2014)

In addition, at adipose tissue level, the fatty acids involved in triacylglycerol synthesis can be transported via triacylglycerol-rich lipoproteins (chylomicrons and low density lipoproteins (LDL)). The enzyme lipoprotein lipase (LPL), which is located in the luminal surface of endothelial cells, hydrolyses lipoprotein triacylglycerols into two free fatty acids and one monoacylglycerol. In this case, PPAR γ is the transcriptional factor that controls the expression of this enzyme. AMPK plays again an important role because its phosphorylation is related with the decrease of fatty acid synthesis and the activation of ACC and also with the downregulation of SREBP-1c through the mammalian target of rapamycin (mTOR) and Liver X Receptor alfa (LXR α) (Zhang et al., 2009).

Resveratrol showed anti-lipogenic effects at both hepatic and adipose levels (**Table 6**). Treatment with this bioactive compound at low doses down regulates, PPAR γ , SREBP-1c, ACC, FAS gene expression in adipocyte cells (Chen et al., 2011; Liang et al., 2013). A similar effect has also been described for pterostilbene (5-10 μ M) that is able to reduce the G6PDH activity in 3T3-L1 cells (Hsu et al., 2012). The ability of stilbenes to increase the phosphorylation of APMK and their capacity to bond to the ketoacyl reductase (KR) domain of FAS are two mechanisms implicated in the anti-lipogenic effects of these bioactives (Chen et al., 2011; Li et al., 2016; Liang et al., 2013).

Alternatively, by using different hepatocytes cell lines, resveratrol and oxyresveratrol are able to decrease the hepatic lipogenesis by suppression of SREBP-1, FAS, ACC and stearoyl- CoA desaturase-1 (SCD-1) (Choi et al., 2014; Gnoni and Paglialonga, 2009; Jin et al., 2013; Lee et al., 2018). This last protein is a rate-limiting enzyme that catalyzes the synthesis of monounsaturated fatty acids and it is essential for the assembly of VLDL particles, which transport triacylglycerol (TG) from liver to adipose tissue and other sites (Li et al., 2009). AMPK activation and the inhibition of LXR α which activate the SREBP-1 have been the molecular pathways related (Choi et al., 2014; Jin et al., 2013; Lee et al., 2018).

Lipolysis and β -oxidation occur when the body requires energy. In adipocytes the TG are metabolized giving rise to glycerol and fatty acids by the catabolic action of adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL). HSL activation

depends on protein kinase A (PKA) phosphorylation, which is mediated via the accumulation of cAMP (Duncan et al., 2007). With regard to the lipolytic effects of stilbenes, resveratrol was the main compound studied. Some published articles proved that resveratrol, at different doses (0.03-400 μ M), is able to enhance the free fatty acid and the glycerol release by increasing HSL and ATGL expression in some cellular models (bovine intramuscular adipocytes, 3T3-L1 and SGBS adipocytes) (Chang et al., 2016; Lee et al., 2018; Rayalam et al., 2007). Interestingly, resveratrol had also synergistic action with genistein, CLA and epinephrine enhancing its lipolytic capacity (Lasa et al., 2011; Rayalam et al., 2007; Szkudelska et al., 2009).

An increment of β -oxidation capacity in adipocyte cells was demonstrated by resveratrol and oxyresveratrol. In fact, the upregulation of carnitine palmitoyltransferase 1 (CPT-1) which is necessary for mitochondrial import of fatty acids and the repression of receptor interacting protein 140 (rip 140), a suppressor of oxidative metabolism, was involved (Lee et al., 2018; Mercader et al., 2011).

Finally, another important pathway related to fat metabolism is thermogenesis that is literally defined as heat production. The white adipose tissue (WAT) and the brown adipose tissue (BAT) are the main types of adipose tissues with antagonistic functions. WAT stores the excess of energy in the form of TG and BAT is specialized in heat production. BAT is specialized in dissipate energy thanks to the high number of mitochondria. Mitochondria membranes contain high amounts of Uncoupling protein 1 (UCP-1), an inner membrane protein that uncouples the electron transport chain from ATP synthesis resulting in energy dissipation rather than ATP synthesis (Madden, 2017). Under certain conditions (e.g. extreme low temperature or β -3 adrenergic agonist) the number of mitochondria increased drastically; this process is named "browning" and the type of WAT is called "beige" (Bartelt and Heeren, 2014). Thus, an increase of BAT thermogenesis is considered nowadays as a potential strategy to reduce obesity.

Several recent *in vitro* studies have revealed that resveratrol (10 μ M) can activate the "browning" process. In particular an increase of UCP-1 expression in stromal vascular cells from interscapular WAT and BAT has been observed (Wang et al., 2015; Wang et al., 2017). Beyond this, these studies have also demonstrated an increase of expression of others transcriptional factors of brown adipogenic program as PR

domain-containing 16 (PRDM16) that leads to the activation of mitochondrial biogenesis. This effect was related with AMPK signaling pathway activation. Other papers showed that resveratrol at 100 μ M concentration up-regulated the gene expression of some mitochondrial activity regulators such as SIRT3 that influences the mitochondrial function by reducing membrane potential, mitofusin 2 (Mfn2) that participates in mitochondrial fusion in mammalian cells, and ATPase family AAA Domain-containing protein 3 (ATAD3), a protein that regulates mitochondrial biogenesis (Li et al., 2016; Rayalam et al., 2008).

Anti-obesity in vivo effects of stilbenes

Regarding the *in vivo* effects and similarly with the *in vitro* studies the most examined stilbene molecule was resveratrol.

Several animal models were used to investigate the anti-obesity effects of stilbenes, mice (Swiss, C57BL/6J, FVB/N, CD1, kunming) and rats (Sprague-Dawley, Wistar, Zucker (*fa/fa*)) being the most commonly used. In addition, other animal models such as *Megalobrama amblycephala* (fish) or *Caenorhabditis elegans* were used. Experimentally, these species were treated with standard (SD), high fat (HFD), high protein (HPD), high fructose corn syrup (HFCSD) diets supplemented with stilbenes (between 1 to 300 mg/kg/day) during different periods of time varying from some hours to 20 weeks (**Table 7**). This large numbers of conditions (animal model, dose, diet and period of treatment) makes sometimes difficult the understanding of results found by these studies. Nevertheless, several studies showed that resveratrol is able to mitigate the body weight gain in different animal models. In some instances, even a 50% reduction was described (Choi et al., 2014; Jeon et al., 2014; Majumdar et al., 2014; Mendes et al., 2016; Qiao et al., 2014).

In addition to weight reduction, an attenuation of lipid deposition on internal adipose tissues such as epididymal and intraperitoneal tissues was observed after resveratrol treatment (De Almeida Pinheiro et al., 2017; Gómez-Zorita et al., 2013; Jeon et al., 2014; Zhang et al., 2018). This fact was related to the capacity of resveratrol to control and reduce the adipogenesis process. Particularly, resveratrol (0.4%) reversed the HFD-induced up-regulation of key adipogenic genes such as PPAR γ , C/EBP α , SREBP-1c, FAS, LPL, aP2, and leptin in mice adipose tissues (Kim et al., 2011). The results reported by Kim et al. highlighted that galanin-mediated signaling molecules are also implicated on the anti-adipogenesis effects of resveratrol. Galanin is a neuropeptide that plays a role in food intake and its circulating serum levels are elevated in obese individuals (Kim and Park, 2010). Resveratrol has demonstrated its capacity to significantly reverse the HFD-induced up-regulation of galanin and its receptors along with increased expression and/or activation of downstream molecules related to adipogenesis, such as Protein Kinase C Delta (PKC δ), cyclin D (Cyc-D), transcriptional factor E2F1, and Extracellular Receptor Kinase (ERK) (Kim et al., 2011).

Table 7. *In vivo* effects of resveratrol, other stilbenes and combination of stilbenes and other bioactives on adipogenesis, apoptosis, lipogenesis, lipolysis and thermogenesis.

Stilbene and dose	Diet/Model	Effect	Reference
Resveratrol 30 mg/Kg/day 8 weeks	SD Swiss mice	↓Lipogenesis (adipose tissue) ↓Weight of the epididymal adipose tissue ↑Insulin sensitivity, glucose tolerance	(De Almeida Pinheiro et al., 2017)
Resveratrol 300 mg/Kg/day 8 weeks	HFD,HPD FVB/N mice	↓Adipogenesis (adipose tissue), lipogenesis ↓Body weight, body adiposity, adipose tissue weight, adipocyte area, total cholesterol ↓PPAR γ , SREBP-1c, ACC and FAS ↑HDL-cholesterol	(Mendes et al., 2016)
Resveratrol 1-30 mg/Kg/day 10 weeks	HFD C57BL/6C mice	↓Weight gain ↓Lipid deposition in adipose tissues (subcutaneous and epididymal) and liver ↓Fat droplet accumulation	(Chang et al., 2016)
Resveratrol 30 mg/Kg/day 60 days	HFD FVB/N mice	↓Lipogenesis (hepatic), adipogenesis, inflammation ↓Body fat, total cholesterol, triacylglycerol, transaminases, insulin plasma level ↓TNF- α , IL-6, and NF-kB ↓ACC, PPAR γ , SREBP-1c ↑ SIRT1	(Andrade et al., 2014)
Resveratrol 50 mg/Kg/day 7 weeks	HFCSD Sprague-Dawley rats	↓Body weight, BMI ↓Cholesterol, TG and FFA ↓Hypertrophy ↓Macrophage infiltration	(Majumdar et al., 2014)

Resveratrol 200 mg/Kg/day 12 weeks	HFD Kunming mice	<p>↓Adipogenesis (adipose tissue), lipogenesis</p> <p>↓Body and visceral adipose weights, blood glucose and lipid levels</p> <p>↓LPL, SCD-1, PAR-g ACC1, and FAS</p> <p>↑Fiaf</p> <p>↑<i>Bacteroidetes</i> to <i>Firmicutes</i> ratios</p> <p>↓<i>Enterococcus faecalis</i></p> <p>↑<i>Bifidobacterium</i> , <i>Lactobacillus</i></p>	(Qiao et al., 2014)
Resveratrol 0.4 % 10 weeks	HFD C57BL/6J mice	<p>↓Adipogenesis (adipose tissue), lipogenesis</p> <p>↓ Body, visceral fat-pad weights</p> <p>↓TG, FFA, total cholesterol, glucose</p> <p>↓TNF-α, MCP1</p> <p>↓ Galanin-mediated signaling molecules: GalR1/2, PKCδ, Cyc-D, E2F1, p-ERK</p> <p>↓PPARγ, C/EBPα, SREBP-1c, FAS, LPL, aP2, leptin</p> <p>↓ TNF-α, IFNα, IFNβ, IL-6</p>	(Kim et al., 2011)
Resveratrol 0.04, 0.36, and 1.08% 10 weeks	HFD Blunt snout bream (<i>Megalobrama amblycephala</i>)	<p>↓ FA uptake, lipogenesis, fatty acid synthesis</p> <p>↓Body mass, whole body fat, intraperitoneal fat</p> <p>↓TG, glucose, and abnormalities of ALT and AST</p> <p>↑SIRT1, ATGL, CPT1, MTTP, Glucose transporter 2 (GLUT2)</p> <p>↓LPL, SREBP-1c, PPARγ, ATP citrate lyase (ACLY) ,Glucokinase (GCK) and sodium-dependent glucose transporter 1 (SGLT1)</p>	(Zhang et al., 2018)
Resveratrol 20 mg/Kg/day 8 weeks	HFD Wistar rats	<p>↓Lipogenesis (hepatic), β-oxidation, pancreatic activity</p> <p>↑Insulin sensitivity</p> <p>↓Proteolytic cleavage of SREBPs-1 and SREBPs-2</p> <p>↓FFA beta-oxidation (CPT-1, UCP-2)</p>	(Khaleel et al., 2018)

Resveratrol 30 mg/Kg/day 6 weeks	HFD Wistar rats	↓Lipogenesis (adipose tissue) ↑MicroRNA of PPAR γ , HSL, and sp1 ↓SREBP-1, FAS	(Gracia et al., 2016)
Resveratrol 28-46 mg/Kg/day 12 weeks	HFCSD Wistar rats	↓Lipogenesis (hepatic) ↓Body weight ↓FAS, SREBP-1c ↑IRS-1, SIRT1, eNOS	(Sadi et al., 2015)
Resveratrol 15 and 45 mg/Kg/day 2 days	HFD ICR mice	↓Lipogenesis (hepatic) ↓SREBP-1c, FAS ↓Hepatic TG ↑pAMPK	(Choi et al., 2014)
Resveratrol 0.1% 11 weeks	HFD C57BL/6 mice	↓Lipogenesis (hepatic) ↓Body weight ↓PPAR α , SREBP-1c, ACC1, FAS ↑SIRT 1	(Sun et al., 2015)
Resveratrol 0.02% 12 weeks	Atherogenic diet Apo-E deficient mice	↑FA oxidation (liver and WAT), lipogenesis ↓Body weight, epididymal fat weight ↓Total cholesterol, LDL, ↑HDL ↓TG (plasma and hepatic) ↓G6PDH, FAS, ME, PAP	(Jeon et al., 2014)
Resveratrol 30 mg/kg/day 6 weeks	HFD Wistar rats	↓Adipogenesis, lipogenesis (adipose tissue) ↓Rev-Erba	(Alberdi et al., 2013)
Resveratrol	HFD	↓Lipogenesis (liver)↑FA oxidation	(Alberdi et al., 2013)

30 mg/Kg/day 6 weeks	Sprague-Dawley rats	↓Liver fat accumulation ↑CPT-1a, ACO ↓ACC ↑AMPK, PGC1α	
Resveratrol 15 o 45 mg/kg/day 6 weeks	SD Zucker (<i>fa/fa</i>) rat	↑FA oxidation ↓Liver weight and TG content ↑CPT-1a, ACO ↓NEFA, ALP, AST, GOT	(Gómez-Zorita et al., 2012)
Resveratrol 0.0125% 8 weeks	Atherogenic diet C57BL6/J mice	↓Lipogenesis (hepatic), lipolysis ↓Body and liver weight ↓Total cholesterol plasma, hepatic TG ↓Fdf1, FAS, PPARγ ↓Aldh1b1 ↓Apoa4, Cd36 ↑SIRT1	(Ahn et al., 2008)
Resveratrol 100 mg/Kg/day 10 weeks	HFD Wistar rats	↓Lipogenesis (hepatic) ↓Lipid accumulation ↑Fasting plasma insulin ↑pAMPK ↓SREBP-1, FAS	(Shang et al., 2008)
Resveratrol 0.04%	SD SAMP10 mice	↓lipogenesis ↑lipolysis ↑ACC	(Shiozaki et al., 2011)

20 weeks			↓Lipid droplets ↑mitochondria ↑Mitochondrial ATP synthase, subunit Mn superoxide dismutase (SOD2)	
Resveratrol 0.1% 4 weeks	HFD CD1 mice		↑Browning, FA oxidation ↓Body weight, Iwat index (iwat mass/body weight), adipocyte diameter ↓TG ↑UCP-1, PRDM16, Cytochrome C ↑AMPK α	(Wang et al., 2015)
Resveratrol 15 mg/Kg/day oral 6 weeks	SD Zucker (<i>fa/fa</i>) rat		↓FA uptake, lipolysis ↓Weight of adipose tissues ↓G6PDH, ACC, LPL ↑HSL ↓Cluster of differentiation 36 ↓TNF- α , MCP1, CRP, IL-6, NF- κ B	(Gómez-Zorita et al., 2013)

Combinations and dose	Diet/Model	Effect	Reference
Resveratrol (15 mg/kg/day) + quercetin (30 mg/kg/day) 6 weeks	HFD Wistar rats	↓Weight of all the fat depots (epididymal, perirenal, mesenteris and subcutaneous) ↓HR-LPL ↓ACC ↑ATGL	(Arias et al., 2016)
Resveratrol (30 mg/kg/day) + Conjugated linoleic acid (CLA) (0.5 %)	HFD Wistar rats	↓HR-LPL, FAS ↑HSL Non synergic effects	(Arias et al., 2014)
Resveratrol (0.003% drinking solution) + phenelzine (0.02%) 12 weeks	SD VHFD C57Bl6/J mice	↓Epididymal fat depots No effect fat body composition Non synergic effects	(Carpéné et al., 2014)
Resveratrol (50 mg/Kg/day) + Melatonin (3 mg/kg/day) 7 weeks	ST and HFCSD Sprague-Dawley rats	↓Body weight, body mass index, feed efficiency, serum glucose, cholesterol, TG, free fatty acids ↓hypertrophy of adipocytes ↓macrophage infiltration	(Majumdar et al., 2014)
Resveratrol (50 mg/Kg/day) + Metformine (200 mg/kg/day) 10 days	HFD ICR mice	↓ lipolysis (↓PKA/HSL ↓accumulation cAMP) ↓ FFA, glycerol	(Zhao et al., 2016)

Other stilbenes and dose	Diet/Model	Effect	Reference
Piceatannol 50 and 100 μ M 48 hours	S and HG conditions <i>Caenorhabditis elegans</i>	↓Fat accumulation ↓SBP-1, FAS ↑HOSL-1	(Shen et al., 2017)
Piceatannol 0.1 and 0.25% 18 weeks	HFD C57BL/6 mice	↓Body weight (0.25% piceatannol) cholesterol, LDL, HDL levels, and blood glucose ↓Weight of liver, spleen, perigonadal and retroperitoneal fat ↑pAMPK, pACC ↓ C/EBP α , PPAR γ , CPT-1 ↑ <i>Firmicutes</i> , <i>Lactobacillus</i> ↓ <i>Bacteroidetes</i>	(Tung et al., 2016)
Ptrostilbene 15 and 30 mg/kg/day 6 weeks	SD Zucker (<i>fa/fa</i>) rats	↑UCP-1, CPT-1b, PPAR γ , nuclear respiratory factor 1 (Nrf1), Cox2, citrate synthase (CS) ↑PPAR α , p38 MAPK, glucose ↑GLUT4	(Aguirre et al., 2016)
Pterostilbene 300 mg/kg/day 4 weeks	SD OLETF rats	↓ Abdominal white adipose tissue ↑Oxygen consumption and energy expenditure ↑UCP-1, FAS, leptin	(Nagao et al., 2017)
Pterostilbene 15 or 30 mg/kg/day 6 weeks	HFD Wistar rats	↓Adipose tissue mass ↓ME, FAS ↓ACC ↑AMPK ↓ME, G6PDH (liver) ↑CPT-1a, acyl-coenzyme A oxidase (liver)	(Gómez-Zorita et al., 2014)

Oxyresveratrol 0.25 and 0.5 % 8 weeks	High fat diet fed mice C57BL/6 mice	<hr/> ↓Insulin resistance, hyperglycemia, hepatic steatosis ↓Body weight, liver weight, visceral ↓ G6PDH, SREBP-1c, FAS, C/EBP ↑ AMPK2 ↑GLUT4/IRS1 ↑AMPK α , PGC1 α , SIRT1	(Tan et al., 2017)
Oxyresveratrol 10 and 30 mg/kg/day 4 weeks	SD HFD C57BL/6 mice	↓Fasting glucose, cholesterol, LDL cholesterol	(Lee et al., 2018)

There is evidence that microbiota has an important role in obesity. In fact, the presence of certain bacteria and overall, the relative proportions and composition of microbial communities is key for energy homeostasis (Tennyson and Friedman, 2008). Although this research line is recent and remains not fully explored, some studies were published indicating that in obese people exposed to low calorie diet, *Bacteroidetes* level increased while *Firmicutes* levels decreased (Ley et al., 2006). In addition, it seems that gut microbiota can abolish the expression of fasting-induced adipose factor (Fiaf) that belongs to the family of fibrinogen/angiopoietin-like proteins. When Fiaf was suppressed an increase of LPL and a higher deposition of TG in adipose tissues have been observed (Bäckhed et al., 2004). For this reason, the study of gut microbiota in obesity research is gaining more and more relevance.

HFD was related with microbiota dysbiosis (promoting the growth of endotoxin producers) producing a decrease of *Lactobacillus* and an increase of *Enterococcus faecalis*. In this context it is worth mentioning that resveratrol long term (12 weeks) treatment in Kunming mice's demonstrated that this stilbene increased the *Bacteroidetes* to *Firmicutes* ratio and diminished the growth of *Enterococcus faecalis* (Qiao et al., 2014). Similarly, the numbers of *Lactobacillus* and *Bifidobacterium* were significantly increased. At intestinal level, this work showed that resveratrol increases the Fiaf expression which can be linked to the suppression of LPL and SCD-1 expressions in the liver, and with the expression of adipogenesis/ lipogenesis genes (PPAR γ , ACC1, and FAS) in adipose tissues (Qiao et al., 2014).

Likewise, some of the above-mentioned papers proved that resveratrol can also be able to reduce lipogenesis process. A reduction of expression levels of FAS, ACC, SCD-1, and G6PDH, and therefore, a suppression of fatty acid uptake and TG synthesis at adipose and hepatic level has been observed (**Table 7**). Furthermore, resveratrol causes the increase of GLUT2 mRNA expression allowing to restore the normal glucose fluxes induced by HFD (Zhang et al., 2018). Another recent work showed for the first time that resveratrol modifies the microRNA (miRNA) profile in WAT. Actually, the reduction of protein levels of FAS, SREBP-1 and SP-1 (acts together with SREBP1 to synergistically activate the promoter of FAS) has been linked to the up-regulation of miR-539-5p (Gracia et al., 2016).

An inhibition of adipogenesis by resveratrol at hepatic level has also been evidenced. Some works have mentioned a decrease of mRNA expression of PPAR γ , SREBP-1c and FAS after resveratrol supplementation with HFD, HFSCD or atherogenic diets (Ahn et al., 2008; Alberdi et al., 2013; Andrade et al., 2014; Sadi et al., 2015; Shang et al., 2008). Furthermore, this action is mediated by the activation of AMPK/SIRT1 axis (Alberdi et al., 2013; Shang et al., 2008). Indeed, Shang et al. showed an increase of 164% of AMPK phosphorylation level in liver after the oral administration of resveratrol (100 mg/Kg/day) during 10 weeks (Shang et al., 2008). Furthermore, higher gene and protein expressions levels of a great number of insulin-signaling molecules including IR, IRS-1/2, eNOS as well as SIRT1 have been outlined after resveratrol supplementation (Sadi et al., 2015).

One of the key developments in obesity research is the recognition that this disorder is also characterized by chronic mild inflammation. Indeed, an increase of circulating levels of inflammatory markers in obese people such as CRP (C-reactive protein), tumor necrosis factor alpha (TNF- α), interleukin (IL)-6 and IL-18, was described (Festa et al., 2001; Monteiro and Azevedo, 2010). In this context, some papers indicated that a reduction of inflammatory markers (TNF- α , interferon IFN α , IFN β , IL-6, monocyte chemoattractant protein 1 (MCP-1), and CRP) was observed in the adipose tissues of mice and rat after resveratrol oral treatment, relating that with the repression of Toll-like receptor (TLR)2- and TLR4-mediated pro-inflammatory signaling cascades (as Nuclear Factor Kappa B (NF- κ B) pathway) in the adipose tissues of mice and rat after resveratrol oral treatment (Gómez-Zorita et al., 2013; Kim et al., 2011). This action was also observed at hepatic level and evidenced by the reduction of TNF- α , IL-1 β , IL-6 and NF- κ B expression in liver in association with the up-regulation of SIRT1 in mice treated with an HFD and resveratrol (Andrade et al., 2014).

Additionally, resveratrol is able to improve fatty acid oxidation in liver and WAT. Particularly, Enzyme activities involved in fatty acid oxidation as CPT-1a, a marker of mitochondrial oxidation, and acyl-coenzyme A oxidase (ACO), a marker of peroxisome oxidation, were significantly increased by resveratrol in liver (Alberdi et al., 2013; Gómez-Zorita et al., 2012). It was furthermore demonstrated by other authors that the increase of expression levels of both CPT-1 and UCP-2 may exert a protective effect of resveratrol on mitochondrial dysfunction not only by inhibition of fatty acid oxidation

but also in association with reactive oxygen species (ROS) generation (Khaleel et al., 2018). By using a fish animal model (*M. amblycephala*), the supplementation with different doses of resveratrol (0.04, 0.36 and 1.08%) resulted in a significant reduction of ATGL, CPT-1 and the microsomal triglyceride transfer protein (MTTP) implying up-regulation of lipolysis and β -oxidation (Zhang et al., 2018). Finally, an augmentation of HSL, without affecting the ATGL levels, was observed in Zucker (*fa/fa*) rats after oral supplementation of 15 mg/Kg/day of resveratrol during 6 weeks (Gómez-Zorita et al., 2013).

Finally, a strengthening of “browning” and mitochondrial biogenesis has been observed by increasing protein contents of UCP1, PRDM16, and Cytochrome C along with an increase of AMPK in CD1 mice treated with 0.1% of resveratrol (Wang et al., 2015).

As for *in vitro* tests, the combination of resveratrol with other polyphenols, bioactives and drugs has been object of certain studies (**Table 7**). Two works published by the same research group have proved that the mixture of resveratrol (15 mg/Kg/day) and quercetin (30 mg/Kg/day) for a 6 weeks period of time decreases the weight of liver and all the fats depots. This effect has been related with the reduction of LPL activity and ACC and the increase of ATGL and CPT-1 (Arias et al., 2016; Arias et al., 2015). However, non-synergistic effects have been found when resveratrol is combined with conjugated linoleic acid (CLA) (Arias et al., 2014). Melatonin is a neurohormone related with the circadian rhythms but also a bioactive found ubiquously in several foods and also in wine (Hornedo-Ortega et al., 2016). The supplementation of melatonin (3 mg/kg/day) with resveratrol in ovariectomized rats reduced the body weight by 16% and body mass index (BMI) by 19%. Moreover, this combination is able to reduce the insulin resistance and macrophage infiltration in liver (Majumdar et al., 2014). Lastly, lipolysis was attenuated through prevention of PKA/HSL activation by decreasing the accumulation of cAMP via preserving phosphodiesterase 3B (PDE3B) by the mixture of metformin (oral antidiabetic drug) and resveratrol (Zhao et al., 2016).

With regard to other stilbenes, some *in vivo* studies pointed out that piceatannol, pterostilbene and oxyresveratrol are interesting molecules to combat obesity (**Table 7**). Starting with the first one and by utilizing *C. elegans* as *in vivo* model, authors demonstrated that this compound reduced the fat accumulation induced by high glucose conditions; an attenuation of SBP-1 (encode SREBP-1c) and FAS and a

reduction on HOSL-1 expression (encodes HSL) prove that piceatannol can prevent the lipid synthesis and stimulate the lipolysis (Shen et al., 2017). Furthermore, Tung et al. showed that piceatannol (0.1 and 0.25%; 18 weeks) can decrease the C/EBP α , PPAR γ , FAS and CPT-1 and consequently promote the mitochondrial FA, oxidation and lipid accumulation in adipocytes and liver. As was displayed for resveratrol, this compound can also alter the composition of gut microbiota specifically by increasing of *Firmicutes/Lactobacillus* and decreasing *Bacteroidetes* (Tung et al., 2016).

Pterostilbene at low doses (15 and 30 mg/Kg/day) can interfere in *de novo* lipogenesis at adipose and hepatic level by reducing the activity of: ME, FAS, ACC, G6PDH, CPT-1, which is in part explained by the increase of *p*-AMPK levels (Gómez-Zorita et al., 2014). This paper highlighted that pterostilbene was more efficient than resveratrol at a dose of 15 mg/kg/day while at 30 mg/kg/day both stilbenes had similar responses. This fact can be explained, by the higher bioavailability of pterostilbene (Gómez-Zorita et al., 2014). Using the same doses of pterostilbene, a thermogenic and oxidative capacity by increasing of brown adipose tissue markers (UCP-1, CPT-1b, nuclear respiratory factor 1 (Nrf1), cyclooxygenase 2 (COX2)) was observed (Aguirre et al., 2016).

Finally, an improvement of insulin resistance and hyperglycemia accompanied by the reduction in body weight (up to 26%), liver weight (up to 28%), and visceral fat (up to 51%) was observed for oxyresveratrol in C57BL/6 mouse experiments. The down regulation of G6PDH expression was interpreted as the repression of free glucose production in liver. They also observed an increase of GLUT4 and IRS1 that also plays a vital role in intracellular glucose uptake (Tan et al., 2017).

Conclusion

Stilbene concentration in grape and wines can vary substantially. In fact, up to 20 times more stilbenes can be found depending of the *Vitis vinifera* cultivars. The quantities can also change significantly if the vine has suffered a pathogen infection, or if it has been treated with elicitor or UVC treatments. In wines, the duration of maceration and the activity of yeast can also influence the final amounts of stilbenes. Moreover, the winemaking processes or the wine ageing can be not only affecting the stilbene quantities but also inducing the formation of stilbene oligomers. However and relating with this last one, more studies are needed to evaluate if this process can have a relevant impact on the final product. On the contrary, it is a proven fact that the composition of stilbenes in red wine is much more complex (oligomers) than in white wine and that a greater complexity of stilbenes is observed in wild *V. vinifera* cultivars in comparison with cultivated *V. vinifera*. Until today, more than 30 stilbenes have been identified in grapes and wines using different separation and analytical methodologies. In this context, the liquid chromatography coupled to mass spectrometry is the most used method to determine stilbene compounds due to its sensitivity, rapidity and the less consumption of solvents. In addition, the use of mass spectrometers of high resolution (as QTOF) that provide information about the exact mass and fragments (with 4-5 decimals) and molecular formula, allows more accurate stilbene identification. However, a generalized problem is the lacks of commercial standards (overall of oligomers) that force to express the results in resveratrol or piceid equivalent, and could severally underestimate the oligomeric stilbene content. Regarding anti-obesity properties of stilbenes, a great number of *in vitro* and *in vivo* studies have allowed to demonstrate not only the positive implications of these bioactives but also the underlying mechanisms of the observed effects. In fact, stilbenes can modulate all fat metabolism pathways (adipogenesis, lipogenesis, lipolysis and thermogenesis) by regulating gene and protein expression of receptors and key transcription factors, enzymatic activities or even by diminishing the production of inflammatory markers with a direct implication of different molecular mechanisms such as SIRT1-AMPK-FOXO1 and NF-KB pathways, among others. The understanding of these mechanisms is an essential task since the scientific community seeks the development of more specific dietary recommendations to prevent or combat obesity. It is noteworthy that although

the most studied molecule is resveratrol, other stilbenes are gaining attention as pterostilbene, oxyresveratrol, viniferins and vitisins, some of them showing better anti-obesity activities than resveratrol. In addition the combination of stilbenes with others polyphenols or drugs is also reporting promising results. It is important to emphasize that a great number of published papers use in general high doses, and without reflecting important processes such as absorption and metabolism or even the role of gut microbiota. Furthermore, intervention studies are still necessary in order to prove these beneficial effects in humans.

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Chapitre 3 :

***Propriétés antioxydantes des extraits de pépins et
de pellicules de trois cépages rouges au cours de la
maturité***

Les objectifs de ce chapitre étaient de déterminer l'évolution des teneurs en polyphénols et la capacité antioxydante au cours de la maturation de trois cépages rouges (Merlot, Tannat et Syrah). Les paramètres suivants ont été mesurés : teneurs en polyphénols, flavanols, anthocyanes et propriétés antioxydantes des extraits de pépins et de pellicules (année 2017).

Les méthodes utilisées dans ces travaux ont été :

1. le test de Folin-Ciocalteu pour obtenir la teneur en polyphénols totaux ;
2. l'UPLC-MS pour déterminer les teneurs en flavanols et en anthocyanes ;
3. trois tests spectrophotométriques (ABTS, DPPH et FRAP) afin de déterminer la capacité antioxydante totale ;
4. une méthode électrochimique (voltammétrie cyclique) utilisant des électrodes jetables pour déterminer la capacité antioxydante totale des extraits et caractériser les composés les plus facilement oxydables ;

Les hypothèses étaient :

1. la teneur en polyphénols totaux varie au cours de la maturation et en fonction du tissu (pépins et pellicules).
2. la capacité antioxydante varie au cours de la maturation et en fonction du tissu.
3. les résultats des tests électrochimiques utilisant les électrodes jetables sont corrélés à ceux des tests spectrophotométriques.
4. les teneurs en flavanols et en anthocyanes évoluent au cours de la maturation.
5. l'activité antioxydante est corrélée à la teneur en anthocyanes et flavanols.

Conclusions

1. la teneur en polyphénols varie au cours de la maturation. Les extraits de pépins sont toujours plus riches en polyphénols comparés à ceux des pellicules, avec une teneur plus élevée à la véraison pour les pépins et au stade vert pour les pellicules.

2. la capacité antioxydante suit la même évolution. Celle-ci varie au cours de la maturation du raisin. Elle est plus importante dans les pépins par rapport aux pellicules. L'activité mesurée est maximale à la véraison pour les pépins et au stade vert pour les pellicules.
3. les résultats des tests électrochimiques utilisant les électrodes jetables sont bien corrélés avec ceux des tests spectrophotométriques. On peut considérer la voltammétrie cyclique comme une méthode alternative pour les tests de routine en raison de sa sensibilité, sa rapidité, sa facilité d'utilisation.
4. les teneurs en flavanols et en anthocyanes évoluent au cours de la maturation et diffèrent entre pépins et pellicules. Les flavanols sont présents majoritairement dans les pépins, leur teneur augmente jusqu'à la véraison puis diminue. Dans les pellicules, leur teneur est plus élevée au stade vert. Les anthocyanes, présentes uniquement dans les pellicules, voient leur teneur augmenter de la véraison jusqu'à la maturité.
5. l'activité antioxydante déterminée en utilisant les deux méthodes (spectrophotométriques et électrochimiques) suit la même évolution que la teneur en flavanols. La forte corrélation entre ces composés et la charge anodique du 1^{er} pic de la voltammétrie cyclique confirme que les flavanols sont de puissants antioxydants.

Cette étude a fait l'objet d'un article scientifique, publié dans le journal *Antioxidants*, sous la référence :

Benbouguerra, N., Richard, T., Saucier, C., & Garcia, F. (2020). Voltammetric Behavior, Flavanol and Anthocyanin Contents, and Antioxidant Capacity of Grape Skins and Seeds during Ripening (*Vitis vinifera* var. Merlot, Tannat, and Syrah). *Antioxidants*, 9(9), 800.

Et ci-après présentée.



Article

Voltammetric Behavior, Flavanol and Anthocyanin Contents, and Antioxidant Capacity of Grape Skins and Seeds during Ripening (*Vitis vinifera* var. Merlot, Tannat, and Syrah)

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Abstract: Skin and seed grape extracts of three red varieties (Merlot, Tannat, and Syrah) at different stages of ripening were studied for their total phenolic content (TPC) by using the Folin-Ciocalteu assay and for their total antioxidant capacity (TAC) by using spectrophotometric and electrochemical assays. Flavanol and anthocyanin compositions were also investigated using Ultra Performance Liquid Chromatography coupled with Mass Spectrometry (UPLC-MS). Results showed that seeds had the highest phenolic content and the highest antioxidant potential compared to skins at all stages of ripening. The highest TPC and TAC values were measured in seeds at close to veraison and veraison ripening stages. In skins, the highest values were found at the green stage, it was in accordance with the flavanols content. The voltammetric measurements were carried out using disposable single walled carbon nanotubes modified screen-printed carbon electrodes (SWCNT-SPCE). Three peaks on voltammograms were obtained at different oxidation potentials. The first anodic peak that oxidized at a low potential describes the oxidation of ortho-dihydroxy phenols and gallate groups, the second peak corresponds to the malvidin anthocyanins oxidation and the second oxidation of flavonoids. The third voltammetric peak could be due to phenolic acids such as *p*-coumaric acid and ferulic acid or the second oxidation of malvidin anthocyanins. The high linear correlation was observed between antioxidant tests and flavanols in skins ($0.86 \leq r \leq 0.94$), while in seeds, '*r*' was higher between electrochemical parameters and flavanols ($0.64 \leq r \leq 0.8$).

Keywords: skins; seeds; *Vitis vinifera*; antioxidant activity; cyclic voltammetry; phenolic compounds

1. Introduction

Vitis vinifera is the most economically important species of grape vine in the world with 78 million tons of grapes production in 2018 (see <http://www.oiv.int/en/oiv-life/oiv-2019-report-on-the-world-vitivinicultural-situation>). Grapes consumed as fresh fruits, juices, and other processed products, contain many phenolic compounds which are mostly located in seeds and skins [1]. These compounds are synthesized in response to various biotic and abiotic stress such as fungal invasion, UV irradiations, ozone, and heavy metal ions [2]. Their content changes depending on the grape variety, soil, climatic conditions, and the ripening stages [3].

Polyphenols are commonly present in the plant kingdom and they bring more and more interest [4]. Phenolic compounds can be divided in two groups, flavonoids and non-flavonoids, according to their carbon skeleton [4]. The flavonoids (C₆-C₃-C₆) are located in both skins and seeds and the anthocyanins

and flavanols are the most abundant compounds [5]. The non-flavonoids such as stilbenes and phenolic acids are found in the skins [6]. The synergy between the various classes of polyphenols increases sample efficiency and activity [7]. Polyphenols protect plants against biotic and abiotic stresses and they are involved in organoleptic and qualitative properties of food and beverages derived from these plants [8]. Many studies have reported their biological activities. They have potent antioxidant capacity [7,9–18]. They may prevent diabetes [19,20], obesity [21–23], cardiovascular [24,25], and neurodegenerative diseases [25,26].

Radical scavenging capacity (DPPH and ABTS) and ferric reducing capacity, which are spectrophotometric assays, are usually used in order to determine the antioxidant capacity of foods and beverages [13]. In the last years, electrochemical techniques have been more widely used as alternative methods due to their sensitivity, rapidity, ease of use, and due to their minimal environmental effects [27]. Among these electrochemical techniques, cyclic voltammetry (CV) was the first and the most commonly used to characterize and determine the total polyphenols and the total antioxidant capacity [27]. The main CV (anodic curve) parameters are:

- The peak current which is proportional to the concentration of antioxidant.
- The peak potential which indicates the type of reductant (the more the oxidation potential is low, the more the reductant is strong and easy to oxidize).
- The charge (area under the curve) is in accordance with the antioxidant capacity of samples [28].

Electrode made of glassy carbon electrode is widely used but recently, carbon nanotubes electrode have become one of the most promising material [29]. This electrode is classified into two categories depending on the number of layers on multi-walled carbon nanotubes (MWCNTs) and single-walled nanotubes (SWCNTs) [29,30]. Actually, disposable screen-printed carbon electrodes modified with carbon nanotubes attract the attention of many researchers because of their numerous advantages including disposability [31], reproducibility, practicality, high sensitivity, the ability to be miniaturized to minimize the consumption of samples, and the low detection limits [32,33].

The aims of this work were:

- To determine the polyphenol content of skin and seed extracts (Merlot, Tannat, and Syrah) during ripening.
- To measure the antioxidant capacity (DPPH, ABTS, and FRAP) of these extracts.
- To determine the cyclic voltammetry behavior of these extracts by using disposable single walled carbon nanotubes electrodes for electrochemical tests.
- To determine the correlations of electrochemical parameters with the other antioxidant assays as well as with the phenolic contents.

2. Materials and Methods

2.1. Chemicals and Reagents

Folin-Ciocalteu reagent, sodium carbonate, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), persulfate de potassium, 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH), sodium acetate trihydrate, ferric chloride, 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), iron(II) sulfate heptahydrate, phloroglucinol, ascorbic acid, sodium acetate, tartaric acid, sodium hydroxide, gallic acid, trolox, catechin, caffeic acid, *trans*-resveratrol, hydrochloric acid, and glacial acetic acid were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). Oenin chloride was obtained from Extrasynthese (Genay, France). Acetonitrile, methanol, and water UPLC-MS were purchased from Biosolve Chimie (Dieuze, France) and trifluoroacetic acid from Carlo Erba Reagents (Peypin, France).

2.2. Samples

Three *V. vinifera* varieties (Merlot, Tannat, and Syrah) were harvested on 2017 at different stages of ripening: Green stage (GS), close to veraison (CV), veraison (V), and maturity (M) (Supplementary

Data, Table S1) from INRAe experimental vineyard (Montpellier, France) (coordinates: 43°37′02.7″ N 3°51′22.3″ E, average annual temperature: 15.85 °C, average annual precipitation: 629 mm (the weather was quite dry), and soil: Gravels and river sand). The whole grapes were stored at −80 °C in plastic bags until polyphenols extraction.

2.3. Samples Preparation

Seeds and skins of thirty Merlot, Tannat, and Syrah berries were manually removed from the pulp. The polyphenols were extracted with 100 mL of acetone/water (70/30 *v/v*) deoxygenated with nitrogen for 5 min. The solutions were filtered through a 0.45 µm filter paper after stirring during 18 h in the dark, and they evaporated in a rotavapor under low pressure at 37 °C. The resulting products were freeze-dried and stored at −20 °C until their use in antioxidant and other analytical assays [34]. Three biological replicates were done. After extraction, skin and seed extracts were weighted (dry weight: DW) and they were stored at −20 °C between 5 and 12 months before being used in the experiments.

2.4. Determination of Phenolic Composition

2.4.1. Flavanols

The assay on flavanols was performed as described by [35]. Briefly, a solution of 0.1 N HCl in MeOH, containing 50 g/L phloroglucinol and 10 g/L ascorbic acid was prepared. Seed and skin grape extracts were dissolved in methanol and reacted for 20 min at 50 °C in this solution, and then combined with five volumes of 40 mM aqueous sodium acetate to stop the reaction.

The UPLC system was a Waters Acquity (Saint-Quentin-en-Yvelines, France), with a photodiode array detector (PDA), LC pump, and an auto sampler. The column used was a reversed phase UPLC with an Acquity UPLC BEH C18 column (2.1 × 50 mm, 1.7 µm particle size) (Saint-Quentin-en Yvelines, France). The method used a binary gradient with mobile phases: Mobile phase A containing 1% *v/v* aqueous trifluoroacetic acid and mobile phase B containing acetonitrile. The 20 min elution method at flow 0.45 mL/min was 0 min 2% B, 8 min 6% B, 14 min 20% B, 14.1 min 99% B, 16 min 99% B, 16.1 min 2% B, and 20 min 2% B. The column temperature was 40 °C. Eluting peaks were monitored at 280 nm. The catechin calibration curve was used. Results were expressed as mg/g of DW.

2.4.2. Anthocyanins

Skin grape extracts were solubilized in MeOH/water (80/20 *v/v*) at an appropriate concentration then injected directly after filtration as described previously with some modifications [36].

The conditions of the chromatographic apparatus are the same as those mentioned in experimental Section 2.4.1. The column temperature was set at 50 °C. The 40 min elution method at flow 0.25 mL/min was 0 min 1% B, 5 min 8.8% B, 30 min 20.6% B, 30.5 min 96% B, 34 min 96% B, 34.1 min 1% B, and 40 min 1% B. The detection was monitored at 520 nm. The malvidin-3-O-glucoside calibration curve was used. Results were expressed as mg malvidin-3-O-glucoside equivalent (M3GE)/g of DW.

2.5. Determination of Total Phenolic Content

Skin and seed grape extracts (dry weight) were solubilized in methanol at a concentration of 5 g/L. The same solution was used to determine the total phenolic content (TPC) and total antioxidant capacity (TAC) assays. To measure the absorbance, an Agilent Cary 60 UV-Vis spectrophotometer (Santa Clara, CA, USA) connected to the Cary win UV software was used.

The Folin-Ciocalteu method was used to determine the total phenolic content (TPC) [3,13,37]. Twenty µL of the diluted extract (see Section 2.5) and 100 µL of Folin-Ciocalteu reagent were added to 1.58 mL of water. After 30 s, 300 µL of sodium carbonate solution (20%) were added; the reaction mixture was thoroughly shaken and left for 120 min in the dark at room temperature (20 °C). Then, the absorbance was measured at 700 nm against the blank (sample was replaced by the methanol).

The gallic acid calibration curve was used to determine the concentration of phenolic compounds in samples. The results were expressed as mg gallic acid equivalent (GAE)/g DW.

2.6. Determination of Antioxidant Capacities

2.6.1. Radical Scavenging Activity: DPPH• Assay

DPPH antioxidant capacity was determined according to a published protocol [38]. Fifty μL of diluted extract (see Section 2.5) was added to 1.95 mL of a DPPH (6×10^{-5} M) methanolic solution. After 30 min of incubation in the dark at room temperature (20 °C), the absorbance was measured at 515 nm. The trolox calibration curve was used. The results were expressed as $\mu\text{mol TE/g DW}$.

2.6.2. Radical Scavenging Activity: ABTS Assay

ABTS antioxidant capacity was determined according to [39]. To generate ABTS• radical, 20 mL of ABTS solution (7 mM) was added to 20 mL of a potassium persulfate solution (2.45 mM). The mixture was incubated at room temperature in the dark all night. The stock solution was diluted with water/ethanol (50/50 *v/v*) to an absorbance of 0.7 ± 0.02 at 734 nm. One hundred μL of diluted extract (see Section 2.5) was mixed with 1 mL of ABTS• solution. After 10 min, the absorbance was measured at 735 nm. The trolox calibration curve was used. Results were expressed as $\mu\text{mol TE/g DW}$.

2.6.3. Ferric-Reducing Antioxidant Power: FRAP Assay

FRAP antioxidant capacity was determined according to reference [40]. Fifty μL of diluted extract (see Section 2.5) and 150 μL of distilled water were added to 1.5 mL freshly prepared FRAP reagent (mixture of 10 volumes of a 300 mmol/L acetate buffer pH 3.6 with 1 volume of 10 mmol/L TPTZ in 40 mmol/L hydrochloric acid and 1 volume of 20 mmol/L ferric chloride). The solution was incubated at 37 °C for 4 min. Absorbance was measured at 593 nm. The $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ calibration curve was used. Results were expressed as $\text{mmol Fe}^{+2}\text{E/g DW}$.

2.6.4. Electrochemical Apparatus and Measurements

Electrochemical measurements were carried out with potentiostat/galvanostat, Autolab PGSTAT 302N controlled by the Nova 2.1.3 software (Metrohm, Switzerland) in the personal computer (Supplementary Data, Figure S1). Tartaric acid buffer (3.3 mM tartaric acid adjusted with 1 M NaOH to obtain a pH 3.6) was used to prepare standard phenolic compounds solutions as well as diluted extracts (see Section 2.5) at appropriate concentrations (100 mg/L for skins and 20 mg/L for seeds). The scan rate was 100 mV/s.

Disk Electrode

Voltammetric measurements were carried out in a standard three-electrode electrochemical cell using an Ag/AgCl (KCl, 3 M) reference electrode, platinum counter electrode, and a glassy carbon electrode (GCE) of 3 mm diameter (Metrohm, Switzerland) as working electrode. Before each test, the working electrode surface was carefully polished with 3 μm alumina powder, then washed with purified water and cleaned for 5 min in an ultrasonic bath.

Disposable Single-Walled Carbon Nanotubes Electrodes

Single-walled carbon nanotubes electrodes (4 mm diameter, Dropsens, Spain) were also used in a three-electrode configuration comprising single-walled carbon nanotubes (SWCNTs-SPCE) with a silver reference electrode and carbon counter electrode. An aliquot of 200 μL of a solution of standard polyphenols or samples was cast onto the surface of the electrode, and the electrochemical measurements were performed immediately.

2.7. Statistical Analysis

The ANOVA and correlation tests were calculated by using the XLSTAT software (Addinsoft version 19.02, Paris, France). A Tukey test was carried out and where p -values < 0.05 was considered as significant. Pearson's correlation coefficient was carried out for the determination of correlations between the different antioxidant assays (spectrophotometric and electrochemistry) and between the antioxidant assays and phenolic composition (anthocyanins and flavanols).

3. Results and Discussion

3.1. Flavanol and Anthocyanin Content of Skin and Seed Grape Extracts during Ripening

The results of the evolution of total flavanols and anthocyanins content in skin and seed grape extracts are presented in Table 1.

Table 1. Phenolic composition of skin and seed grape extracts of the studied varieties at different stages of ripening.

	Skins		Seed
	Flavanols (mg/g DW)	Anthocyanins (mg M3GE/g DW)	Flavanols (mg/g DW)
Merlot			
Green stage	199 ± 19 ^a	ND	545 ± 9 ^a
Close to veraison	124 ± 16 ^b	2 ± 1 ^c	598 ± 30 ^a
Veraison	45 ± 9 ^c	17 ± 1 ^b	437 ± 17 ^b
Maturity	42 ± 2 ^c	22 ± 1 ^a	329 ± 24 ^c
Tannat			
Green stage	224 ± 40 ^a	ND	424 ± 27 ^{bc}
Close to veraison	166 ± 74 ^a	ND	530 ± 16 ^a
Veraison	31 ± 5 ^b	10 ± 3 ^b	469 ± 21 ^b
Maturity	19 ± 2 ^b	36 ± 2 ^a	382 ± 23 ^c
Syrah			
Green stage	198 ± 34 ^a	ND	496 ± 19 ^{ab}
Close to veraison	100 ± 26 ^b	3 ± 1 ^c	532 ± 21 ^a
Veraison	40 ± 7 ^c	28 ± 1 ^a	439 ± 32 ^b
Maturity	18 ± 1 ^c	14 ± 1.0 ^b	201 ± 41 ^c

Values represent means of triplicate determination ± SD. Different letters indicate the significant differences between stages according to Tukey's test, $p < 0.05$. DW: Dry Weight; MG3E: Malvidin-3-O-Glucoside Equivalent.

3.1.1. Flavanols

Skins

For the three varieties, the highest flavanol content was determined at the green stage then it decreased significantly until maturity. It declined from 224 mg/g DW at the green stage to 19 mg/g DW at maturity in Tannat grape extracts. A similar evolution was shown in the literature [41,42]. On the opposite, an increase of flavanols content during ripening was also observed in other study [43].

Seeds

The highest content of flavanols was reached at close to veraison compared to the green stage and the maturity for all varieties. It increased from 424 mg/g DW at the green stage to 530 mg/g DW at close to veraison, then it declined significantly to 382 mg/g DW at maturity in seed Tannat grape extracts. This evolution was in accordance with a previous study [41]. The decline of flavanols content was explained by the oxidation of these compounds after veraison [44].

Flavanols are present in both skins and seeds at all stages of ripening with an abundance in seeds [27,45]. It has been shown that in Syrah skins at maturity the content was about 250 mg/g DW

and about 455 mg/g DW in seeds [46]. There is an important variability in the literature concerning the phenolic composition content due to the extraction solutions, methods, and unit used to express results.

3.1.2. Anthocyanins

The anthocyanin synthesis started at close to veraison and they accumulated until maturity in Merlot and Syrah skins, in Tannat skins, the anthocyanin synthesis started at veraison. The content increased from 2 mg M3GE/g DW to 22 mg M3GE/g DW at maturity in skin Merlot extracts. A similar evolution was reported in the literature [41–43,47–50]. In the case of Syrah, the anthocyanins content decreased at maturity from 28 to 14 mg M3GE/g DW, this decline may be due to the degradation of anthocyanins by the peroxidases and glycosidases present in skins [47].

Anthocyanins, the pigmented compounds, are present only in skin red grapes. As flavanols, the anthocyanins content differs considerably in the literature. It increases from 1.80 to 3.81 mg/g DW in Tannat skins [51] and it is about 86.68 mg/g DW at maturity in another study [16]. As mentioned previously, the anthocyanins content is also greatly affected by weather, climatic conditions, soil conditions, cultivars, irrigation [49], temperature, and light [52].

3.2. Electrochemical Behavior of Polyphenol Standards and Skin and Seed Extracts for Various Cultivars at Different Stages of Ripening

3.2.1. Electrochemical Behavior of Standard Polyphenols

Cyclic voltammograms of polyphenol standards in tartaric acid buffer (pH 3.6) at glassy carbon electrode (GCE) in a potential range from 0 to 1100 mV (vs. Ag/AgCl-KCl 3M) and at single-walled nanotubes (SWCNT) in a potential range from 0 to 800 mV (vs. Ag) are illustrated in Figure 1 and peak potentials are given in Table 2. For caffeic acid, only one anodic peak was present. This peak corresponds to the oxidation of the *ortho*-diphenols to form the corresponding *o*-quinone. The potential values for the concentration 0.1 mM are 445 mV (vs. Ag/AgCl-KCl 3M) for GCE and 139 mV (vs. Ag) for SWCNT. Two peaks were observed for catechin and gallic acid at 0.1 mM. With GCE (vs. Ag/AgCl-KCl 3M), the voltage values were 483/826 mV for gallic acid and 472/766 mV for catechin. With SWCNT (vs. Ag), the voltage values were 132/468 mV and 122/465 mV for catechin and gallic acid, respectively. For both catechin and gallic acid, the first anodic peak correspond to the oxidation of the hydroxyl groups on the B-ring to quinone [53]. This oxidation was reversible generating cathodic peak in the negative scan for caffeic acid and catechin. The second peak corresponds to the oxidation of the hydroxyl group on the C-ring of catechin and can also correspond to the oxidation of the third phenol group adjacent to the *ortho*-diphenol group in gallic acid which is in agreement with previous results [54]. Other phenolic standards characterized corresponding to the anthocyanins and the flavonols are present mostly in skin grapes. Oenin chloride and rutin at 0.1 mM presented two anodic peaks at 377/669 mV and at 201/460 mV with SWCNT (vs. Ag), respectively, and at 652/987 mV and at 260/898 mV with GCE (vs. Ag/AgCl-KCl 3M), respectively (Figure 1).

The classification obtained considering only the first peak potential for the studied standards at the same concentration (0.1 mM) by increasing potential was: Gallic acid (122 mV) < catechin (132 mV) < caffeic acid (139 mV) < rutin (201 mV) < oenin chloride (377 mV) was found [55] since catechin, caffeic, and gallic acid oxidized at lower potential.

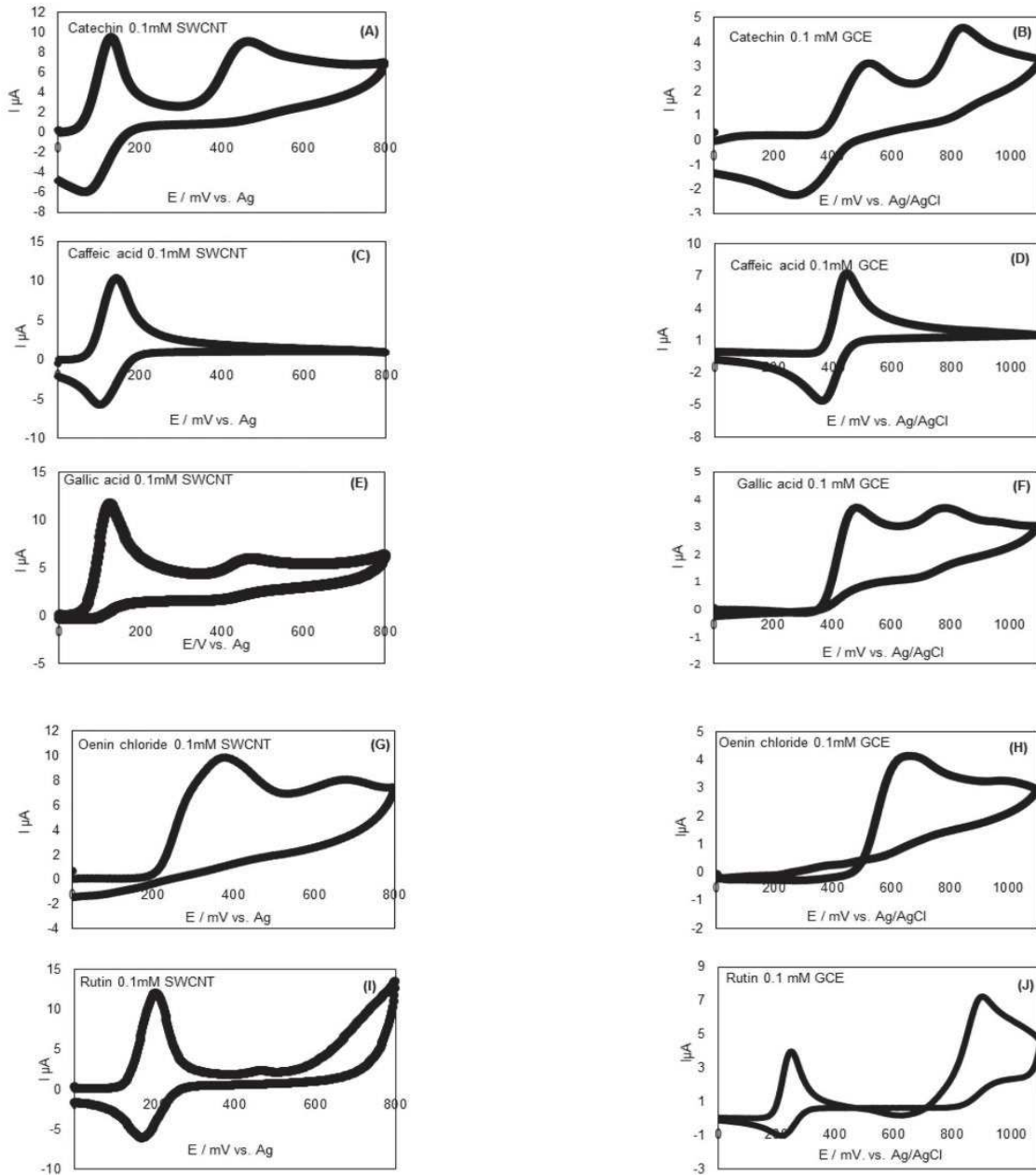


Figure 1. Cyclic voltammograms of catechin with SWCNT (A) and GCE (B), caffeic acid with SWCNT (C) and GCE (D), gallic acid with SWCNT (E) and GCE (F), oenin chloride with SWCNT(G) and GCE (H), rutin with SWCNT (I) and with GCE (J) at a concentration of 0.1 mM (blank subtracted). GCE: Glassy Carbon Electrode; SWCNT-SPCE: Single Walled Carbon Nanotubes modified Screen Printed Carbon Electrodes.

Table 2. Voltammetric behavior of the studied standard polyphenols in tartaric acid buffer (pH 3.6) with SWCNT-SPCE and GCE for a concentration of 0.1 mM.

Standards	Potential (mV)			
	SWCNT-SPCE (vs. Ag)		GCE (vs. Ag/AgCl-KCl 3M)	
	Ep,a1	Ep,a2	Ep,a1	Ep,a2
Catechin	132	468	483	826
Caffeic acid	139	/	445	/
Gallic acid	122	465	472	766
Oenin chloride	377	669	652	987
Rutin	201	460	260	898

GCE: Glassy Carbon Electrode; SWCNT-SPCE: Single Walled Carbon Nanotubes modified Screen Printed Carbon Electrodes.

3.2.2. Electrochemical Characterization of Skins and Seeds

Voltammetric measurements were performed on the extracts of each variety. For all varieties, cyclic voltammograms had three anodic peaks at different potentials depending on grape part (skins or seeds) (Figure 2) and the ripening stage. Syrah grape seed extracts were studied with both types of electrodes, as with SWCNT, three anodic peaks were also obtained with GCE (Figure 2).

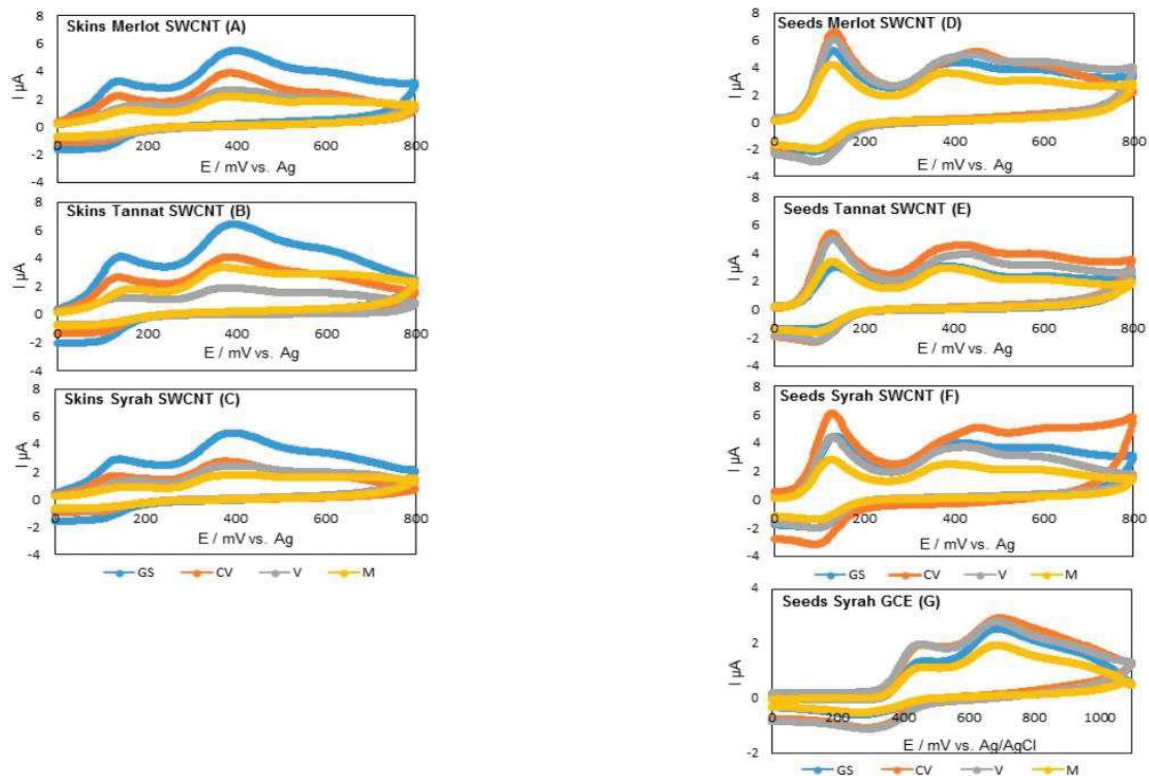


Figure 2. Cyclic voltammograms of skin (A–C) and seed grape extracts (D–F) with SWCNT and those of Syrah seed grape extract (G) with GCE at different stages of ripening (blank subtracted). GS: Green Stage; CV: Close to Veraison; V: Veraison; M: Maturity.

For skin Merlot grape extracts, the first anodic peak was measured at 137, 134, 159, and 157 mV at the green stage, close to veraison, veraison, and maturity, respectively. This peak corresponds to the more oxidizable compounds that oxidized at a low potential as catechin-type flavonoids, including larger oligomeric and polymeric molecules, gallic acid, caffeic acid, and flavonols. The second anodic

peak appeared at 391, 383, 363, and 370 mV at the green stage, close to veraison, veraison, and maturity, respectively. This peak may result from the oxidation of malvidin anthocyanins and stilbene derivatives overlapped with the second oxidation of the catechin flavonoids [56]. The third peak close to 600 mV corresponds to the phenolic acids such as vanillic and *para*-coumaric acid or the second oxidation of malvidin anthocyanins [54]. The same behavior was observed for the two other varieties.

In grape seed extracts, the first anodic peak was obtained at the same potential in all stages of ripening, it was around 130 mV. For Syrah grape extracts for example, the first peak appeared at 136 mV, 127, 129, and 126 mV at the green stage, close to veraison, veraison, and maturity, respectively. The second peak followed the same trend of the first potential with the following potential values for Syrah at 396, 438, 409, and 391 mV for the different stages of ripening. This peak could be attributed to the oxidation of the hydroxyl group on the C-ring of catechin derivatives. The third anodic peak corresponds to the higher oxidation potential compound which produces a peak at around 600 mV [57].

3.3. Total Phenolic Content and Total Antioxidant Capacity by Spectrophotometric and Electrochemical Assays

3.3.1. Total Phenolic Content and Total Antioxidant Capacity by Spectrophotometric Assays

The total phenolic content and the antioxidant capacity of skin and seed grape extracts during ripening were determined using different spectrophotometric methods: Folin-Ciocalteu, DPPH, ABTS as well as FRAP assays, respectively. The results were summarized in Table 3.

Skins

The highest total phenolic content was detected at the green stage of ripening then it decreased significantly at maturity in the three varieties. For example, in Syrah grape extracts, the total phenolic content (TPC) was 212 mg GAE/g DW at the green stage then declined to 63 mg GAE/g DW at maturity.

The antioxidant capacities were measured using a single electron transfer (DPPH, ABTS, and FRAP). The highest total antioxidant capacity (TAC) was found in the green stage compared with maturity. The same evolution was obtained with the three assays on the three varieties. For example, in the skin Syrah grape extract, DPPH values decreased significantly from 853 at the green stage to 557 $\mu\text{mol TE/g DW}$ at maturity, ABTS values from 843 to 357 $\mu\text{mol TE/g DW}$, and FRAP values from 2159 to 780 $\text{mmol Fe}^{+2}\text{E/g DW}$.

Seeds

The TPC in seed grape extracts increased before veraison then decreased after veraison with the highest content at close to veraison for both seed Tannat and Syrah grape extracts, whereas for seed Merlot grape extract, the content decreased significantly from 867 at the green stage to 571 mg GAE/g DW at maturity.

The antioxidant capacity of seed grape extracts followed the same trend with the three antioxidant assays. The antioxidant capacity at close to veraison was higher than that found at the green stage and maturity. Among the samples tested, for seed Syrah grape extracts, the DPPH values increased from 2677 to 2915 $\mu\text{mol TE/g DW}$ then decreased to 1991 $\mu\text{mol TE/g DW}$. ABTS values raised from 1171 to 1325 then declined to 590 $\mu\text{mol TE/g DW}$. FRAP values increased from 3979 to 5386 $\text{mmol Fe}^{+2}\text{E/g DW}$ then decreased to 3460 $\text{mmol Fe}^{+2}\text{E/g DW}$.

Several methods were used to determine total phenolic content and antioxidant capacity of samples to take into account not only the composition of the extracts but also the mode of action and the specificity of the antioxidant [58,59]. Due to its ease of use, the Folin-Ciocalteu assay is the common used method to determine the TPC. The principle is the transfer of electrons from phenolic compounds to phosphomolybdic/phosphotungstic complexes [60]. The weakness of this method is the overestimation of the phenolic content due to the lack of specificity [55,60,61] which can react with other compounds particularly aromatic amines, ascorbic acids, and sugars [61]. In addition, the phenolic compounds react with the Folin-Ciocalteu reagent only under the basic conditions [61]. The three colorimetric methods used to determine the antioxidant capacity DPPH, ABTS, and FRAP

are considered as assays based on the electron transfer [58,61]. DPPH assay is an easy method widely used to determine the antioxidant capacity of natural extracts. The drawback of this method is the variation of reaction time with different phenolic compounds. Caffeic acid, for example, reacts quickly with DPPH whereas the catechin reacts slowly. The results obtained with this method differ depending on the time of readings (from 16 min to some hours) [55]. The FRAP assay is a simple, fast, and robust method used in the determination of the concentration of the most easily oxidized compounds [61]. It is based on the ability to reduce Fe^{3+} to Fe^{2+} quantified at 593 nm. Fe(III)/TPTZ reagent is more stable than DPPH^\bullet and gives results in shorter times [55]. The ABTS assay is based on the reduction by an antioxidant of the generated blue/green ABTS^+ [62]. DPPH and ABTS assays are the easiest to implement and yield the most reproducible results [58]. FRAP and DPPH methods are still used as they are the easy and accurate methods to measure the antioxidant activity [60].

The results of this work confirm that the total phenolic content in skins were lower than in seeds [13]. In skins, the highest antioxidant capacity was found at the green stage but a previous study [48] found the highest TAC at maturity. This difference may depend on the extraction method used but also on the protocol of the test. The total polyphenolic content increased when the berry weight decreased in accordance with previous studies [51].

Table 3. TPC and antioxidant capacities of skin and seed grape extracts of the three studied varieties at different stages of ripening by spectrophotometric methods.

	Skins			
	TPC (mg GAE/g DW)	DPPH ($\mu\text{mol TE/g DW}$)	ABTS ($\mu\text{mol TE/g DW}$)	FRAP (mmol $\text{Fe}^{+2}\text{E/g DW}$)
Merlot				
Green stage	280 ± 39 ^a	763 ± 67 ^a	804 ± 37 ^a	2781 ± 186 ^a
Close to veraison	138 ± 12 ^b	575 ± 46 ^b	748 ± 41 ^a	1925 ± 81 ^b
Veraison	82 ± 11 ^b	349 ± 24 ^c	424 ± 11 ^b	1036 ± 114 ^c
Maturity	76 ± 9 ^b	403 ± 28 ^c	527 ± 80 ^b	1180 ± 16 ^c
Tannat				
Green stage	258 ± 21 ^a	932 ± 120 ^a	1211 ± 120 ^a	2704 ± 431 ^a
Close to veraison	188 ± 44 ^b	647 ± 123 ^b	1109 ± 188 ^a	2322 ± 537 ^a
Veraison	72 ± 14 ^c	409 ± 54 ^b	696 ± 79 ^b	805 ± 8 ^b
Maturity	111 ± 9 ^c	528 ± 48 ^b	612 ± 36 ^b	1219 ± 39 ^b
Syrah				
Green stage	212 ± 39 ^a	853 ± 94 ^a	843 ± 124 ^a	2159 ± 432 ^a
Close to veraison	103 ± 21 ^b	674 ± 10 ^{ab}	687 ± 141 ^{ab}	1239 ± 251 ^b
Veraison	85 ± 8 ^b	639 ± 22 ^b	529 ± 88 ^{ab}	851 ± 29 ^b
Maturity	63 ± 8 ^b	557 ± 29 ^b	357 ± 14 ^b	780 ± 62 ^b
Seeds				
Merlot				
Green stage	867 ± 60 ^a	3855 ± 413 ^a	1681 ± 302 ^{ab}	6047 ± 612 ^a
Close to veraison	834 ± 7 ^a	3998 ± 317 ^a	1846 ± 123 ^a	6006 ± 9928 ^a
Veraison	805 ± 92 ^b	3675 ± 172 ^a	1663.92 ± 89 ^{ab}	5436 ± 391 ^a
Maturity	571 ± 23 ^b	2876 ± 300 ^b	1340.8 ± 67 ^b	4683 ± 492 ^a
Tannat				
Green stage	586 ± 57 ^{ab}	3608 ± 201 ^{ab}	1467 ± 266 ^{ab}	4651 ± 726 ^{ab}
Close to veraison	712 ± 69 ^a	3875 ± 118 ^a	1697 ± 45 ^a	5557 ± 503 ^a
Veraison	676 ± 18 ^a	3706 ± 302 ^a	1656 ± 137 ^a	4201 ± 903 ^{ab}
Maturity	489 ± 55 ^b	3114 ± 127 ^b	1240.95 ± 47 ^b	3266 ± 300 ^b
Syrah				
Green stage	556 ± 52 ^{ab}	2677 ± 216 ^{ab}	1171 ± 91 ^a	3979 ± 2115 ^a
Close to veraison	615 ± 21 ^a	2915 ± 467 ^a	1325 ± 46 ^a	5386 ± 742 ^a
Veraison	467 ± 6 ^b	2366 ± 105 ^{ab}	1079 ± 159 ^a	4719 ± 639 ^a
Maturity	454 ± 96 ^b	1991 ± 211 ^b	590 ± 186 ^b	3460 ± 1065 ^a

Values represent means of triplicate determination ± SD. Different letters indicate the significant differences between stages according to Tukey's test, $p < 0.05$. TPC: Total Phenolic Content; DPPH: 1,1-diphenyl-2-picrylhydrazyl free radical; ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)diammonium salt; FRAP: Ferric Reducing Antioxidant Potential; DW: Dry Weight; GAE: Gallic Acid Equivalent; TE: Trolox Equivalent; Fe^{+2}E : Fe^{+2} Equivalent.

3.3.2. Antioxidant Capacity by Electrochemical Method of Skin and Seed Grape Extracts

Different parameters shown in Table 4 allowed the estimation of the antioxidant capacity of extracts by cyclic voltammetry. The total charge $Q_{800\text{mV}}$ corresponds to all oxidizable phenolic compounds that will contribute to the total antioxidant capacity of the extract. $Q_{240\text{mV}}$ represents the electrochemically of the easily oxidizable polyphenols that have consequently the highest antioxidant capacity. $Q_{520\text{mV}}$ estimates the most antioxidant compounds which oxidize until 520 mV (until the second peak of the voltammogram). $Q_{520\text{mV}} - Q_{240\text{mV}}$ corresponds to the compounds that have the lesser antioxidant capacity that oxidize until 520 mV. Finally, $Q_{240\text{mV}}/Q_{800\text{mV}}$ ratio indicates the contribution of the most antioxidant compounds to the total antioxidant capacity of extract.

Skins

$Q_{800\text{mV}}$, $Q_{240\text{mV}}$, and $Q_{520\text{mV}}$ values presented the same evolution for all grape skin extracts. They declined from the green stage to maturity. For example, in Merlot, $Q_{800\text{mV}}$ values decreased from 262 to 118 $\mu\text{C/g DW}$, $Q_{240\text{mV}}$ from 44 to 22 $\mu\text{C/g DW}$, and the antioxidant capacity until 520 mV diminished from 153 to 75 $\mu\text{C/g DW}$. The contribution of the most antioxidant compounds to the total antioxidant capacity was also determined. It followed the same evolution of the other parameters except for Merlot grape extracts where the percentage increased from 17 to 22% then decreased to 19%.

Seeds

Electrochemical parameters of seed grape extracts have the same evolution in the three varieties. They raised from the green stage to close to veraison and veraison then declined until maturity. In Merlot, $Q_{800\text{mV}}$ values increased from 1232 to 1471 $\mu\text{C/g DW}$ then decreased to 1036 $\mu\text{C/g DW}$ at maturity. The antioxidant capacity at 240 mV was about 358 $\mu\text{C/g DW}$ at the green stage, 379 $\mu\text{C/g DW}$ of extract at veraison, and 252 $\mu\text{C/g DW}$ at maturity. Antioxidant capacity of seed extracts until 520 mV has the same trend than the other parameters, it stated from 905 $\mu\text{C/g DW}$ at the green stage then increased to 944 $\mu\text{C/g DW}$ at veraison, and declined to 639 $\mu\text{C/g DW}$ at maturity. The most antioxidant compounds almost contribute with the same percent at all stages of ripening except for Merlot where the percent of $Q_{240\text{mV}}/Q_{800\text{mV}}$ decreased from 30% to 24% at maturity.

Electrochemical parameters in both skins and seeds have the same trend than TPC, TAC values, and flavanol content. The higher TPC and TAC were found in seed grape extracts compared with skin grape extracts, in agreement with the literature [3,45,63]. The percent of $Q_{240\text{mV}}/Q_{800\text{mV}}$ in seed grape extracts was more important than in skin grape extracts. It follows the same evolution of the other parameters in skins except for Merlot, in seeds there is no among differences between stages. At the charge $Q_{240\text{mV}}$ corresponding to the oxidation of flavanols, this result suggests the abundance of these compounds in seeds compared with skins.

3.4. Correlation between TPC, Antioxidant Capacity, and Phenolic Composition

Table 5 shows the Pearson correlation coefficients between TPC, electrochemical parameters, antioxidant assays, and phenolic composition for which: $r < 0.39$ weak correlation, $0.4 < r < 0.69$ moderate correlation $0.7 < r < 0.89$ strong correlation, and $0.9 < r < 1$ very strong correlation [64].

Table 4. Potential of peaks and cumulative peak areas for skins and seeds of Merlot, Tannat, and Syrah during ripening.

		Skins						
		Ep,a1 (mV)	Ep,a2 (mV)	Q _{240mV} ($\mu\text{C/g DW}$)	Q _{520mV} ($\mu\text{C/g DW}$)	Q _{520mV} -Q _{240mV} ($\mu\text{C/g DW}$)	Q _{800mV} ($\mu\text{C/g DW}$)	Q _{240mV} /Q _{800mV} (%)
Merlot	Green stage	137 ± 3 ^b	391 ± 4 ^a	44 ± 6 ^a	153 ± 26 ^a	110 ± 19 ^a	262 ± 55 ^a	17 ± 1 ^{ab}
	Close to veraison	134 ± 2 ^b	383 ± 4 ^a	39 ± 3 ^a	126 ± 14 ^a	87 ± 11 ^a	166 ± 1 ^b	22 ± 3 ^a
	Veraison	159 ± 5 ^a	363 ± 2 ^b	15 ± 1 ^b	57 ± 2 ^b	42 ± 1 ^b	154 ± 4 ^b	13 ± 1 ^b
	Maturity	157 ± 3 ^a	370 ± 1 ^b	22 ± 1 ^b	75 ± 5 ^b	53 ± 4 ^b	118 ± 7 ^b	19 ± 1 ^a
Tannat	Green stage	139 ± 5 ^b	392 ± 5 ^a	65 ± 8 ^a	211 ± 20 ^a	145 ± 13 ^a	315 ± 36 ^a	21 ± 1 ^a
	Close to veraison	133 ± 1 ^b	383 ± 2 ^b	42 ± 10 ^b	134 ± 37 ^b	92 ± 27 ^b	215 ± 5 ^b	18 ± 6 ^a
	Veraison	130 ± 9 ^b	356 ± 3 ^c	21 ± 6 ^c	64 ± 18 ^c	43 ± 12 ^c	154 ± 24 ^b	16 ± 8 ^a
	Maturity	164 ± 2 ^a	362 ± 1 ^c	27 ± 5 ^{bc}	105 ± 16 ^{bc}	78 ± 11 ^{bc}	174 ± 3 ^b	18 ± 5 ^a
Syrah	Green stage	137 ± 1 ^b	383 ± 1 ^a	36 ± 5 ^a	119 ± 21 ^a	83 ± 16 ^a	172 ± 27 ^a	21 ± 1 ^a
	Close to veraison	126 ± 2 ^c	377 ± 3 ^{ab}	29 ± 5 ^{ab}	91 ± 18 ^{ab}	62 ± 13 ^{ab}	152 ± 11 ^a	18 ± 3 ^{ab}
	Veraison	160 ± 5 ^a	362 ± 3 ^{bc}	21 ± 1 ^b	77 ± 4 ^{ab}	56 ± 3 ^{ab}	141 ± 6 ^a	16 ± 1 ^{ab}
	Maturity	141 ± 3 ^b	359 ± 2 ^c	16 ± 3 ^b	59 ± 5 ^b	42 ± 3 ^b	129 ± 1 ^a	14 ± 2 ^b
		Seeds						
Merlot	Green stage	129 ± 4 ^a	390 ± 8 ^{bc}	358 ± 36 ^a	905 ± 90 ^a	547 ± 54 ^a	1232 ± 152 ^{bc}	30 ± 3.64 ^a
	Close to veraison	133 ± 3 ^a	449 ± 4 ^a	393 ± 24 ^a	958 ± 53 ^a	565 ± 29 ^a	1407 ± 35 ^{ab}	28 ± 1 ^{ab}
	Veraison	132 ± 2 ^a	397 ± 1 ^b	379 ± 8 ^a	944 ± 23 ^a	564 ± 15 ^a	1471 ± 65 ^a	26 ± 0.63 ^{ab}
	Maturity	128 ± 1 ^a	380 ± 3 ^c	252 ± 19 ^b	639 ± 56 ^b	387 ± 39 ^b	1036 ± 94 ^c	24 ± 0.47 ^b
Tannat	Green stage	135 ± 3 ^a	377 ± 6 ^b	206 ± 29 ^b	555 ± 64 ^b	349 ± 35 ^b	808 ± 112 ^b	26 ± 1.63 ^a
	Close to veraison	128 ± 1 ^a	392 ± 6 ^{ab}	319 ± 22 ^a	827 ± 49 ^a	508 ± 28 ^a	1313 ± 52 ^a	24 ± 1.08 ^a
	Veraison	129 ± 3 ^a	418 ± 20 ^a	302 ± 16 ^a	746 ± 49 ^a	444 ± 33 ^a	1112 ± 162 ^{ab}	27 ± 2.45 ^a
	Maturity	129 ± 4 ^a	379 ± 2 ^b	216 ± 7 ^b	532 ± 20 ^b	316 ± 16 ^b	813 ± 61 ^b	27 ± 1.14 ^a
Syrah	Green stage	136 ± 3 ^a	397 ± 5 ^c	302 ± 18 ^b	724 ± 36 ^b	516 ± 179 ^{ab}	1165 ± 26 ^{ab}	26 ± 2.24 ^a
	Close to veraison	127 ± 2 ^b	438 ± 6 ^a	388 ± 24 ^a	937 ± 75 ^a	549 ± 50 ^a	1497 ± 15 ^a	23 ± 0.33 ^a
	Veraison	129 ± 1 ^b	409 ± 4 ^b	268 ± 20 ^b	691 ± 80 ^b	424 ± 30 ^{ab}	1082 ± 44 ^{ab}	25 ± 0.84 ^a
	Maturity	126 ± 4 ^b	391 ± 4 ^c	177 ± 33 ^c	463 ± 93 ^c	286 ± 60 ^b	818 ± 42 ^b	24 ± 2.6 ^a

Values represent means of triplicate determination ± SD. Different letters indicate the significant differences between stages according to Tukey's test, $p < 0.05$. DW: Dry weight.

Table 5. Pearson's correlation coefficients of antioxidant capacity using spectrophotometric tests, electrochemical parameters, flavanols, and anthocyanins.

Skins										
	Folin	DPPH	ABTS	FRAP	Q _{240mV}	Q _{520mV}	Q _{520mV} -Q _{240mV}	Q _{800mV}	Flavanols	Anthocyanins
Folin	1	0.83	0.80	0.94	0.88	0.90	0.90	0.84	0.93	-0.62
DPPH	0.83	1	0.75	0.79	0.81	0.82	0.81	0.69	0.86	-0.55
ABTS	0.80	0.75	1	0.84	0.89	0.86	0.85	0.69	0.86	-0.62
FRAP	0.94	0.79	0.84	1	0.86	0.87	0.87	0.75	0.94	-0.68
Q _{240mV}	0.88	0.81	0.89	0.86	1	0.99	0.98	0.84	0.87	-0.60
Q _{520mV}	0.90	0.82	0.86	0.87	0.99	1	1.00	0.85	0.86	-0.53
Q _{520mV} -Q _{240mV}	0.90	0.81	0.85	0.87	0.98	1.00	1	0.85	0.85	-0.49
Q _{800mV}	0.84	0.69	0.69	0.75	0.84	0.85	0.85	1	0.72	-0.50
Flavanols	0.93	0.86	0.86	0.94	0.87	0.86	0.85	0.72	1	-0.77
Anthocyanins	-0.62	-0.55	-0.62	-0.68	-0.60	-0.53	-0.49	-0.50	-0.77	1
Seeds										
	Folin	DPPH	ABTS	FRAP	Q _{240mV}	Q _{520mV}	Q _{520mV} -Q _{240mV}	Q _{800mV}	Flavanols	
Folin	1	0.78	0.77	0.67	0.76	0.79	0.66	0.60	0.67	
DPPH	0.78	1	0.92	0.44	0.56	0.59	0.56	0.41	0.66	
ABTS	0.77	0.92	1	0.56	0.66	0.69	0.62	0.49	0.71	
FRAP	0.67	0.44	0.56	1	0.62	0.66	0.41	0.51	0.58	
Q _{240mV}	0.76	0.56	0.66	0.62	1	0.99	0.89	0.88	0.80	
Q _{520mV}	0.79	0.59	0.69	0.66	0.99	1	0.90	0.88	0.80	
Q _{520mV} -Q _{240mV}	0.66	0.56	0.62	0.41	0.89	0.90	1	0.79	0.74	
Q _{800mV}	0.60	0.41	0.49	0.51	0.88	0.88	0.79	1	0.64	
Flavanols	0.67	0.66	0.71	0.58	0.80	0.80	0.74	0.64	1	

In skins, a strong correlation was found between TPC and electrochemical parameters ($r = 0.88$ vs. $Q_{240\text{mV}}$, $r = 0.90$ vs. $Q_{520\text{mV}}$, and $r = 0.84$ vs. $Q_{800\text{mV}}$). It was shown in the literature that TPC is significantly correlated with electrochemical responses [65], especially with cumulative response up to relatively high potentials [32]. In this study, TPC was better correlated with $Q_{240\text{mV}}$ than $Q_{800\text{mV}}$. Colorimetric antioxidant assays (DPPH, ABTS, and FRAP) were strongly correlated with all electrochemical parameters. The best correlation was found with $Q_{240\text{mV}}$ ($r = 0.81$ vs. DPPH, $r = 0.89$ vs. ABTS, and $r = 0.86$ vs. FRAP) than with $Q_{800\text{mV}}$ ($r = 0.69$ vs. DPPH, $r = 0.69$ vs. ABTS, and $r = 0.75$ vs. FRAP). The methods used are well correlated because they are all based on electron transfer from antioxidant to oxidized compounds [32]. Flavanols content were well correlated with colorimetric assays ($r = 0.93$ vs. Folin-Ciocalteu, $r = 0.86$ vs. DPPH, $r = 0.86$ vs. ABTS, $r = 0.94$ vs. FRAP) as well as electrochemical parameters ($r = 0.87$ vs. $Q_{240\text{mV}}$, $r = 0.72$ vs. $Q_{800\text{mV}}$). The strong correlation between flavanols and $Q_{240\text{mV}}$ compared with $Q_{800\text{mV}}$ indicates that these compounds are the easiest antioxidant compounds that oxidized at a low potential (240 mV). A negative correlation between anthocyanins and the antioxidant tests have been shown, this result is an agreement with a previous study [59].

In seed grape extracts, the best correlation was found between flavanols and electrochemical parameters ($r = 0.80$ vs. $Q_{240\text{mV}}$, $r = 0.80$ vs. $Q_{520\text{mV}}$, and $r = 0.64$ vs. $Q_{800\text{mV}}$) than with spectrophotometric methods ($r = 0.67$ vs. Folin, $r = 0.66$ vs. DPPH, $r = 0.71$ vs. ABTS, and $r = 0.58$ vs. FRAP). A strong correlation was observed between Folin-Ciocalteu, DPPH, and ABTS ($r = 0.78$ vs. DPPH and $r = 0.77$ vs. ABTS) whereas a moderate correlation was found between Folin-Ciocalteu and FRAP ($r = 0.67$). Contrary to skin grape extracts, in seed grape extracts, FRAP have the lowest correlation with all assays compared with the other colorimetric methods. This result illustrates the specificity of each assay and the variability of phenolic composition between skin and seed grape extracts.

The antioxidant capacity was mainly related to the TPC of extracts in accordance with previous results [9,12–14,58,62,66,67] and especially to the flavanols content [14]. The antioxidant capacity of polyphenols is mainly linked to their structures, compounds that have more than one aromatic ring, more than one hydroxyl groups in different positions are able to have a highest antioxidant capacity. This may explain the variability of Pearson correlation between the different methods and between skins and seeds.

Principal Components Analysis (PCA)

Figure 3 shows the Biplot graphic that represents the association of the phenolic composition with the antioxidant assays on skin and seed grapes extracts during ripening. The first two principal components explained 94.2% of the total variability. The first axis accounted for 88.6% and the second axis only for 5.6%. From the Biplot, skin grape extracts are separated in the left side from seed grape extracts in the right side.

For skin grape extracts, the stages of maturity were well separated depending mainly on the content of anthocyanins, flavanols, as well as antioxidant capacity, down the stages before veraison (have the highest flavanols content and antioxidant capacity) and up the stages from veraison to maturity (beginning of synthesis and accumulation of anthocyanins, low antioxidant capacity, and flavanols content). For seed grape extracts, it is more difficult to separate the different stages of maturity because the variables are very close.

Flavanols were compounds with the highest positive contribution to the antioxidant capacity, while the anthocyanins were the highest negative contribution in the three varieties studied. As it can be seen in Figure 3, the content of flavanols and the antioxidant capacity were higher in seed than in skin grape extracts.

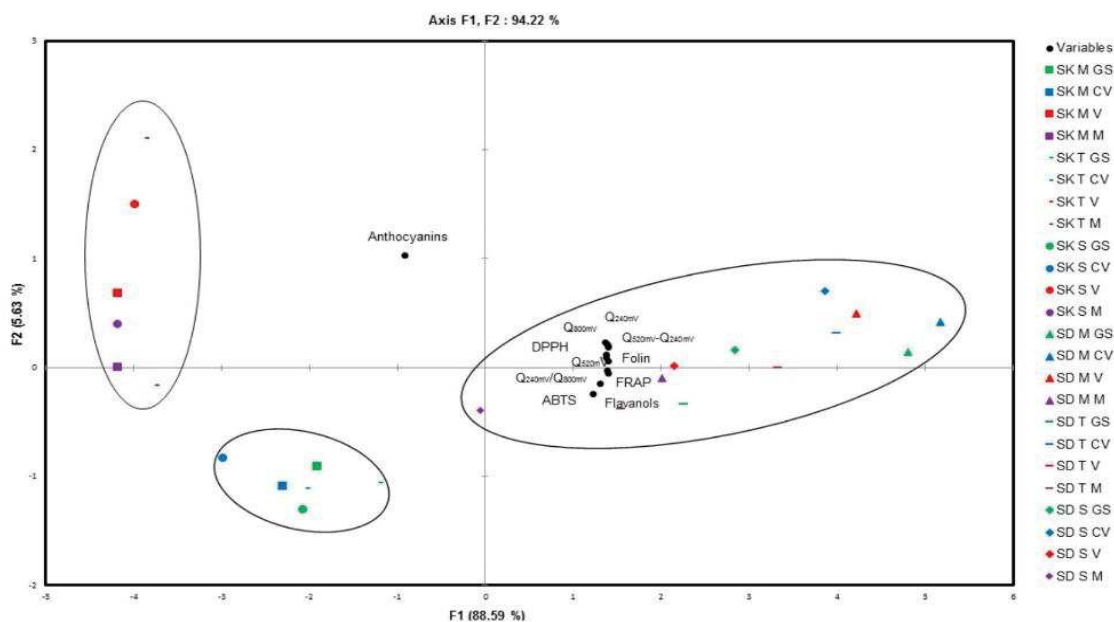


Figure 3. Biplot of the first PCs obtained from PCA for seeds (SD) and skins (SK) of three grape varieties Merlot (M), Tannat (T), and Syrah (S) at different stages of ripening (green stage (GS), close to veraison (CV), veraison (V), and maturity (M)).

4. Conclusions

Total phenolic content, antioxidant capacity, flavanol, and anthocyanin contents of grape skin and seed extracts of three red grape varieties were studied at different stages of ripening. At all stages of ripening, the total phenolic content was higher in seed than in skin grape extracts. The green stage had the highest total phenolic content in grape skin extracts, whereas in grape seed extracts, they were the close to veraison and the veraison that had the highest content.

To measure the antioxidant capacity of extracts, different colorimetric methods were used (DPPH, ABTS, and FRAP) in addition to cyclic voltammetry. In skin grape extracts, the total antioxidant capacity was higher at the green stage than at maturity, in seed grape extracts, they were the close to veraison and the veraison that had the highest content with all assays. Generally, stages that had the highest phenolic content presented also the highest antioxidant capacity.

The correlation between electrochemical results using disposable electrodes and the colorimetric assays indicates that electrochemical assays can be considered as an alternative to these routine tests in the determination and the characterization of the antioxidant capacity in a short time.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2076-3921/9/9/800/s1>. Table S1: Dates corresponding to the different stages of ripening for the three varieties Merlot, Tannat, and Syrah; Figure S1: The experimental electrochemical set up using GCE (A) and SWCNT (B) electrodes.

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Chapitre 4 :

Propriétés antioxydantes et anti-inflammatoires des extraits de pellicules de 3 cépages rouges au cours de la maturation

Les objectifs de ce chapitre étaient de déterminer la capacité antioxydante et anti-inflammatoire des extraits de pellicules de 3 cépages rouges (année 2018) et de leurs teneurs en polyphénols totaux, flavanols, flavonols, anthocyanes et stilbènes.

Les méthodes utilisées dans ces travaux étaient :

1. folin-Ciocalteu pour obtenir la teneur en polyphénols totaux ;
2. dpsh pour déterminer la capacité antioxydante totale ;
3. tests cellulaires pour déterminer la capacité anti-inflammatoire ;
4. l'UHPLC-MS pour déterminer la teneur des flavanols, anthocyanes, stilbènes et flavonols ;

Les hypothèses

1. la teneur en polyphénols des extraits de pellicules varie au cours de la maturation.
2. la capacité antioxydante des extraits de pellicules évolue au cours de la maturation.
3. la capacité anti-inflammatoire des extraits de pellicules se change au cours de la maturation.
4. la teneur en flavanols, flavonols, anthocyanes et stilbènes varie au cours de la maturation.

Conclusions

1. la teneur en polyphénols varie au cours de la maturation, la teneur la plus élevée est déterminée dans le stade vert.
2. la capacité antioxydante évolue au cours de la maturation, elle est plus importante dans le stade vert.
3. la capacité anti-inflammatoire des extraits de pellicules varie au cours de la maturation. Elle est plus importante dans le stade vert puis, elle diminue jusqu'à la maturité.
4. la teneur en stilbènes et en anthocyanes augmente tandis que l'évolution de la teneur en flavonols et flavonols change d'un cépage à l'autre.

Cette étude a fait l'objet d'un article scientifique, sera soumis dans le journal *agriculture and food chemistry*

Et ci-après présenté

31 **1. Introduction**

32 The cultivation of the vine is one of the largest crops in the world. Recent data reported
33 that in 2018, 7.4 million hectares was the global area under vines destined for the
34 production of wine grapes, table grapes or dried grapes. Specifically, the production of
35 wine from *Vitis vinifera* grapes reached 292 millions of hectoliters in 2018. *V. vinifera*
36 species are characteristic for their great diversity. In fact, the actual number of *V.*
37 *vinifera* varieties is estimated at 6 000.¹

38 Polyphenolic compounds are secondary metabolites with undeniable demonstrated
39 biological properties.^{2,3} These compounds are usually divided in two important families:
40 flavonoids (flavan-3-ols, procyanidins, flavonols, anthocyanins) and non-flavonoids
41 (phenolic acids and stilbenes).⁴ Grapes are especially rich in these compounds, and
42 the polyphenolic content and composition varies substantially depending of grape
43 variety, stage of ripening, temperature, soil, fungal infection or radiation and soil
44 salinity.^{5,6} Moreover, polyphenolic compounds are determinant for wine quality, due to
45 their contribution to sensory properties such as color, taste, mouthfeel, flavor,
46 astringency, and bitterness.⁷ In general, red grape skins are initially rich in flavan-3-ols
47 and procyanidins at pre-veraison state whereas at veraison and maturity stages
48 anthocyanins are the major compounds. However, the greater quantities of stilbenes
49 were found at maturity stage.⁸⁻¹⁰

50 The consumption of moderate quantities of red wine, pattern characteristic of the
51 Mediterranean countries, and their richness in polyphenols has been broadly
52 associated to the health benefits of this dietary pattern.^{11,12} Consequently, the study of
53 polyphenolics from grapes and red wines have enthralled the attention of scientists to
54 define their chemical composition and their properties concerning human health.
55 Indeed, grape polyphenols have evidenced beneficial effects to prevent
56 neurodegenerative, cardiovascular and metabolic disorders and certain types of
57 cancers.^{13,14} Oxidative stress and inflammation are important pathological hallmarks
58 related with the development and progression of several diseases.¹⁵ Therefore, in
59 order to counteract this reaction, there is a growing interest to identify grape anti-
60 inflammatory and antioxidant compounds.

61 In this study, the polyphenolic characterization of flavan-3-ols, procyanidins, flavonols,
62 anthocyanins and stilbenes by UHPLC-QqQ-MS and UPLC-PAD of skins from *V.*

63 *vinifera* Merlot, Tannat and Syrah red grape varieties cultivated in the south of France
64 at different stages of ripening on 2018 (before veraison, veraison and maturity) were
65 evaluated. In addition, the anti-inflammatory and the antioxidant potential of extracts in
66 LPS-stimulated macrophages (RAW 264.7 cells) were determined.

67 **2. Materials and Methods**

68 **2.1. Chemicals and reagents**

69 Folin Ciocalteu phenol reagent, sodium carbonate, 1,1-diphenyl-2-picrylhydrazyl
70 (DPPH), Trolox, phloroglucinol, ascorbic acid, tartaric acid, sodium hydroxide,
71 hydrochloric acid, lipopolysaccharide (LPS), RPMI and DMEM mediums, fetal bovine
72 serum (FBS), Griess reagent, 2',7'-dichlorodihydrofluorescein diacetate acetyl (DCFH₂-
73 DA), 3-(4,5-dimethylethiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), dimethyl
74 sulfoxide (DMSO), glutamine, gallic acid, catechin, malvidin-3-O-glucoside, *trans*-
75 piceid, *trans*-resveratrol were obtained from Sigma Aldrich (Steinheim, Germany).
76 *Trans*-astringin was purchased by Carbosynth (UK) and *trans*-piceatannol by
77 ChromaDex (USA). Acetonitrile, methanol and water LC-MS were obtained by
78 Biosolve (Dieuze, France) and Trifluoroacetic acid and sodium acetate were
79 purchased by Carlo Erba (Peypin, France).

80 RAW 264.7 cells were provided by ATCC (Manassas, USA). ϵ -viniferin, δ -viniferin, ω -
81 viniferin, pallidol, parthenocissin A, miyabenol C, hopeaphenol and isohopeaphenol
82 were isolated from a grapevine raw shoot in our laboratory. The *cis*-isomers were
83 obtained using Ultraviolet (UV)-C irradiation (254 nm) from *trans*-isomers.

84 **2.2. Grape samples**

85 Three red *V. vinifera* varieties: Merlot, Tannat and Syrah were harvested from the
86 vineyard of INRAe Montpellier (Coordinates: 43°37'02.7"N 3°51'22.3"E, average
87 annual temperature: 16.38° C, average annual precipitation: 1063.5 mm and soil:
88 gravels and river sand) at different stages of ripening: before veraison (18 June 2018),
89 veraison (27 July 2018) and maturity (3 September 2018). Grapes clusters were frozen
90 within an hour after sampling -at 80°C until sample preparation.

91 **2.3. Grape samples preparation and polyphenolic extraction.**

92 Grapes were thawed and pH and °Brix were measured. For this, 50 berries were
93 crushed, the pH was determined by a multi-parameter analyzer (Consort C3010) and

94 the concentration of sugar was determined using a hand-held refractometer (results
95 expressed as °Brix).

96 Skins of 30 grapes of each variety (Merlot, Tannat and Syrah) were manually
97 separated from the pulp. Then, a solid/liquid extraction using 100 mL of acetone/water
98 (70:30; v/v) previously deoxygenated with nitrogen for 5 minutes were performed in
99 order to extract the polyphenolic compounds. After stirring for 18 hours in the dark, the
100 solution was filtered through a 0.45 µm filter paper and evaporated in a rotavapor under
101 low pressure fixed at 37°C. Finally, the obtained extract was freeze-dried and stored
102 at -80°C until further analysis.

103 **2.4. Determination of Total Polyphenol Content (TPC)**

104 The total phenolic content of the extract was determined by the Folin–Ciocalteu
105 method.¹⁶ A stock solution of 5 g/L was prepared by diluting 5 mg of skins extracts in
106 1 mL of methanol. 20 µL of the diluted extract was mixed to 100 µL of diluted Folin-
107 Ciocalteu reagent. Then, 80 µL of sodium carbonate solution (7.5%) were added. The
108 reaction mixture was thoroughly shaken and left for 30 min in the dark at room
109 temperature (20°C). Finally, the absorbance was measured at 760 nm using a
110 microplate reader (FLUOstar Optima, BMG Labtech). A calibration curve of gallic acid
111 (GAE) (0-0.5mg/mL) was used to determine the concentration of polyphenol in
112 samples. The results were expressed as g of gallic acid equivalents (GAE) per g of
113 skin grapes fresh weight (FW).

114 **2.5. Determination of radical scavenging activity (DPPH' assay).**

115 The antioxidant activity of the extract was determined by the DPPH assay, as
116 described earlier with some modifications.¹⁷ A stock solution of 5 g/L was prepared by
117 diluting 5 mg of skins extracts in 1 mL of methanol. Fifty µL of this stock solution was
118 diluted 4 times in methanol and mixed with 150 µL of a DPPH (200 µM) methanolic
119 solution. After 20 minutes of incubation in the dark at room temperature, the
120 absorbance was measured at 517 nm using a microplate reader (FLUOstar Optima,
121 BMG Labtech). A calibration curve of Trolox (TE) (0-300 µM) was used. The results
122 were expressed as µM TE/g of skin grapes fresh weight (FW).

123 **2.6. Analysis of proanthocyanidins following acid catalysis with phloroglucinol.**

124 Proanthocyanidin analyses were performed with a Waters Acquity Ultra Performance
125 Liquid Chromatography (UPLC) system connected to a photodiode array detector
126 (PDA). The proanthocyanidin assay was carried out as described by Kennedy et al.¹⁸
127 A solution of 0.1 N of HCl in methanol, containing 50 g/L phloroglucinol and 10 g/L
128 ascorbic acid was prepared. Samples (10 g/L in methanol) were mixed with this
129 solution for 20 minutes at 50°C, and then combined with 5 volumes of 40 mM aqueous
130 sodium acetate solution to stop the reaction, then filtrated through PTFE 0.45 µm filters
131 and injected. The column used to analyse phloroglucinol adducts was an Acquity
132 UPLC BEH C18 column (2.1 mm x 50 mm, 1.7 µm particle size) that was thermostated
133 at 40°C during the analysis. The injection volume was 7.5 µL and the flow rate set at
134 0.45 mL/min. The mobile phases consisted of solvent A (water/TFA 99:1 v/v) and
135 solvent B (acetonitrile 100%) with the following gradient: 0 min 2% B, 8 min 6% B, 14
136 min 20% B, 16 min 99% B, and 20 min 2% B. Eluting peaks were monitored at 280
137 nm. In order to calculate the mean Degree of Polymerization (mDP), the sum of all
138 subunits (flavan-3-ol monomers and phloroglucinol adducts expressed in millimoles)
139 was divided by the sum of all flavan-3-ols monomers (expressed in millimoles).

140 **2.7. Individual determination of phenolic composition**

141 **2.7.1. Monomeric flavan-3-ols, procyanidins, flavonols and stilbenes.**

142 The monomeric flavan-3-ols, procyanidins, flavonols and stilbenes analysis was
143 performed by UHPLC coupled to a triple quadrupole (QqQ) mass spectrometer
144 (UHPLC-QqQ-MS) based on a previous method.¹⁹ The freeze-dried skin samples and
145 standards were solubilized in methanol/water (1:1; v/v) at an appropriate concentration
146 (20 g/L). The system was composed of an UHPLC system (Agilent Technology 1260
147 Infinity, Agilent Technologies, Santa Clara, CA, United States), hyphenated to an
148 Agilent Technologies 6430 Triple Quadrupole Detector. An Agilent Poroshell 120 EC-
149 C18 column (150 mm × 2.1 mm, 2.7 µm particle size) was used as a stationary phase.
150 The mobile phases consisted of 0.1% formic acid in water as solvent A and 0.1% formic
151 acid in acetonitrile as solvent B, using the following gradient: 5-17.5% B (0-5 min),
152 17.5%-33% B (5-7.5 min), 33% B (7.5-10 min), 33%-40% B (10-15 min), 40%-95% B
153 (15-16 min), 95% B (16-19 min) and 5% B (19-21 min). The flow rate was fixed at 0.3
154 mL/minute and the column temperature was set at 35°C. The injection volume was 4
155 µL. The detection of compounds was in the multiple reaction monitoring (MRM) mode

156 with specific transitions for each compound, and for quantification purposes a
157 calibration curve was prepared (0.05-26 mg/L) with pure standards. All tests were
158 carried out in triplicate and the results were expressed as mg per kg of skin grapes
159 fresh weight (FW).

160 **2.7.2. Anthocyanins**

161 The anthocyanins analyses were carried out in almost the same column, flow rate and
162 solvent used for proanthocyanidin analyses (2.6. Section). Skin extracts were
163 solubilized at 10 g/L in MeOH/eau (80:20; v/v), filtrated through PTFE 0.45 µm filters
164 then injected. The flow rate was fixed at 0.25 mL/minute. The mobile phases consisted
165 of solvent A (water/TFA 99:1 v/v) and solvent B (acetonitrile 100%) scheduled in the
166 following gradient: 0 min 1% B, 5 min 8.8% B, 30 min 20.6% B, 34 min 96% B, 34.1
167 min 1% and 40 min 1% B. Eluting peaks were monitored at 520 nm. A calibration curve
168 of malvidin-3-O-glucoside (0-200 mg/L) was used to quantify anthocyanins in all
169 samples.²⁰

170 **2.8. Cell culture and treatment**

171 RAW 264.7 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM)
172 containing 10% of fetal bovine serum (FBS) and maintained in a humidified incubator
173 at 37°C with 5% of CO₂. Cells were subcultured at a density of 50000 cells per well, in
174 96-well culture plates with 200 µL of culture medium. After 24 hours, cells were
175 incubated with skin extracts (50-300 µg/mL) in RPMI medium supplemented with
176 glutamine (4 mM) in presence or absence of LPS (0.1 µg/mL) (200 µL final volume
177 per well).

178 **2.9. MTT cell viability**

179 MTT reduction was employed as a cell viability indicator, extensively used as a
180 quantitative and consistent colorimetric assay.²¹ After 24 hours of treatment, RAW
181 264.7 cells were incubated with 0.5 mg/mL of MTT during 3 hours at 37°C. The
182 resulting crystals formed were dissolved with 100 µL of DMSO. After 30 minutes of
183 incubation in darkness, the absorbance was measured at a wavelength of 595 nm
184 using a microplate reader (FLUOstar Optima, BMG Labtech).

185 **2.10. Intracellular NO measurement**

186 In the same way and after 24 hours of treatment, 50 μ L of supernatant mixed with 50
 187 μ L of Griess solution. After 15 minutes in darkness, the absorbance was measured at
 188 a wavelength of 550 nm using a microplate reader (FLUOstar Optima, BMG Labtech).
 189 A calibration curve of NO₂ (0-100 mM) was used. Data were expressed as NO
 190 production (μ M) compared with cells treated only with LPS (positive control).

191 **2.11. Intracellular ROS measurement**

192 Generation of intracellular ROS in cells was analyzed using a fluorometric probe:
 193 DCFH₂-DA. After treatment, cells were washed with PBS and then 150 μ L of DCFH₂-
 194 DA (10 μ M) was added. After 30 minutes at 37°C, the fluorescence intensity was
 195 quantified using a spectrofluorometer (FLUOstar Optima, BMG Labtech). The
 196 wavelengths of excitation and emission used to detect ROS were 485 nm and 520 nm,
 197 respectively. All experiments were performed in the dark. Results were given as ROS
 198 production (fluorescence intensity) compared with cells treated only with LPS (positive
 199 control).

200 **2.12. Statistical analysis**

201 The data was subjected to one-way ANOVA test with XLSTAT version 19.02.
 202 Comparison between the different stages of ripening was performed using Tukey's test
 203 and $p < 0.05$ was considered significant.

204 **3. Results and discussion**

205 Grapes of Merlot, Tannat and Syrah cultivars were harvested at different stages of
 206 ripening: June 2018 (Before Veraison; BV), July 2018 (Veraison; V) and September
 207 2018 (Maturity; M). **Table 1** summarizes the °Brix and pH values of samples.

208 **Table 1.** °Brix and pH of Merlot, Tannat and Syrah berries at GS, V and M stages.

Grapes	Merlot		Tannat		Syrah	
	°Brix	pH	°Brix	pH	°Brix	pH
BV	4.37	2.56	4.3	2.57	4.37	2.58
V	8.57	3.34	11.64	2.75	16.97	3.42
M	25.97	3.78	25.84	3.87	25.37	4

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211 **3.1. Total polyphenol content (TPC) and antioxidant activity of skins of Merlot,**
 212 **Tannat and Syrah cultivars at different stages of ripening**

213 **Table 2** summarizes the TPC expressed as g GAE per kg fresh weight (FW) from the
 214 skins of Merlot, Tannat and Syrah grapes at the three different ripening stages (BV; V
 215 and M). The highest TPC was observed at BV stage in all cultivars (18.29-19.16 g
 216 GAE/kg FW). These values decreased at V and M stages for all cultivars. These results
 217 are in agreement with previous works that observed that TPC decreases when the
 218 berry weight increases.^{22,23} The same tendency was observed for antioxidant activity
 219 calculated by DPPH test (expressed as g TE/kg of skins FW) (**Table 2**). In fact, a
 220 decrease between 28-56% on the antioxidant activity was noticed between BV and M.
 221 In conclusion, TPC is in accordance with DPPH, the greater polyphenol concentration
 222 the greater antioxidant activity (**Table 2**).

223 **Table 2.** Total polyphenol content (TPC) and antioxidant activity (measured by DPPH
 224 test) of Merlot, Tannat and Syrah skin samples at BV, V and M.

225

Skins	TPC (g GAE/kg of skins FW)*		
	Merlot	Tannat	Syrah
BV	18.5 ± 2.6 ^a	19.2 ± 7.9 ^a	18.3 ± 1.2 ^a
V	17.0 ± 2.1 ^b	16.7 ± 2.9 ^b	16.9 ± 2.5 ^b
M	13.1 ± 2.6 ^c	16.5 ± 1.7 ^b	16.2 ± 0.8 ^b
Skins	DPPH (g TE/kg of skins FW)**		
	Merlot	Tannat	Syrah
BV	4.6 ± 0.4 ^b	6.0 ± 0.6 ^b	6.5 ± 0.4 ^b
V	3.8 ± 1.3 ^c	4.1 ± 0.9 ^{ab}	5.9 ± 1.1 ^b
M	3.3 ± 0.1 ^a	3.9 ± 0.9 ^a	2.9 ± 0.4 ^a

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227 *TPC expressed as values means ± SE (n=9) of 3 biological replicates × 3 technical
 228 replicates. Different letters indicate the significant differences between stages
 229 according to Tukey's test, p < 0.05.

230

231 **Antioxidant activity expressed as μmol TE/g of skins (DW). Values means ± SE (n=9)
 232 of 3 biological replicates × 3 technical replicates. Different letters indicate the significant
 233 differences between stages of ripening according to Tukey's test, p < 0.05.

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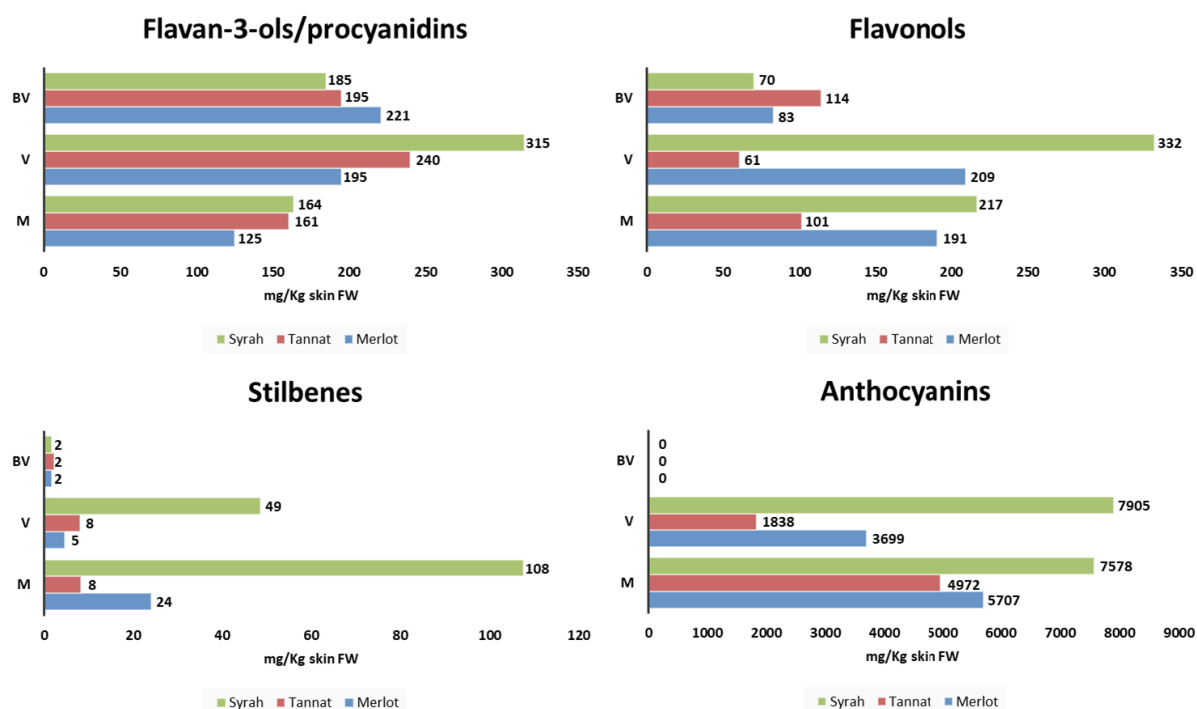
239 **3.2. Individual polyphenolic characterization of skins of Merlot, Tannat and**
 240 **Syrah cultivars at different stages of ripening**

241 **Figure 1** displays the sum of the individual polyphenolic compounds (flavan-3-ols and
 242 procyanidins, flavanols, stilbenes and anthocyanins) expressed as mg/kg of skins FW
 243 of Merlot, Tannat and Syrah extracts at the three ripening stages (BV, V and M). In
 244 addition, **Figure 2**, **Table 3** and **Table 4** summarize the individual polyphenols
 245 identified and quantified in all samples. A total of 41 compounds have been identified:
 246 6 flavan-3-ols and procyanidins; 7 flavanols; 16 stilbenes and 12 anthocyanins.

247 **3.2.1. Monomeric flavan-3-ols and procyanidins**

248 Monomeric Flavan-3-ols and procyanidin are especially relevant in grapes and wine
 249 for their contribution on color stabilization and their astringent and bitter properties.²⁴
 250 They are located in all grape clusters solid parts: skins, seeds and stalks and their
 251 amounts vary during ripening reaching normally their maximal levels around
 252 veraison.²⁵ In our samples the higher levels were observed at V stage (195-315 mg/kg
 253 skin FW). These values diminished significantly at M stage (between 33-48 %) (**Figure**
 254 **1**).

255 **Figure 1.** Sum of the individual flavan-3-ols/procyanidins, flavanols, stilbenes and
 256 anthocyanins of Merlot, Tannat and Syrah skin extracts at different ripening stages.



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259 Regarding flavan-3-ols monomers individually, (+)-catechin is the major compound
260 followed by (-)-epicatechin at BV stage. (+)-catechin quantities diminished
261 considerably during ripening in all varieties (**Table 3**). This tendency has been
262 observed in previously studies for the variety Syrah,²⁶ Tannat,²² and Merlot.²³
263 Interestingly, (-)-epicatechin levels increased from BV to V stage to finally diminished
264 at M stage. In any case, the final amounts of (-)-epicatechin are significantly higher than
265 the initials (BV stage). This observation was also in accordance with other studies.^{22,23}

266 Four B-type procyanidins were identified and quantified in all samples: B1, B2, B3 and
267 B4. Among them, procyanidin B1 (epicatechin-(4 β →8)-catechin) was the predominant
268 with initial values (BV) ranging from 50.77-75.53 mg/kg skins FW. The major levels for
269 this compound were observed at the V stage to finally decrease at the M stage.
270 Important amounts of procyanidin B3 were also present in all samples, which also
271 decreased over the course of maturation in all cultivars and in all ripening stages.
272 Finally, procyanidins B2 and B4 were also present in all samples with levels between
273 4.5 and 9.57 mg/kg of FW (**Table 3**). In accordance with our study, the procyanidin B1
274 was quantified as the major compound in Syrah skin extracts at maturity.²⁶ In a
275 previous study, these four B-type proanthocyanidins have also been quantified in
276 Merlot skins extracts at different maturity stages, however procyanidin B2 was the
277 predominant.²³

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Table 3. Quantification of individual flavan-3-ols, procyanidins, flavonols and stilbenes in skins extracts of Merlot, Tannat and Syrah varieties at different stages of ripening.

	Merlot			Tannat			Syrah		
	BV	V	M	BV	V	M	BV	V	M
Flavan-3-ols/procyanidins*									
(+)-Catechin	107.04±1.48	53.36±0.51	23.95±0.95	114.45±4.66	90.11±5.18	29.43±6.56	97.72±16.66	111.11±0.02	39.82±5.84
(-)-Epicatechin	1.38±0.44	5.30±0.37	5.90±2.47	2.32±0.78	9.85±0.96	7.31±1.50	0.86±0.08	30.94±5.54	8.75±0.30
Procyanidin B1	75.53±3.90	128.23±3.56	71.43±2.14	50.77±0.39	108.18±5.16	105.36±6.09	52.66±4.24	138.63±8.90	89.92±9.03
Procyanidin B2	2.09±0.36	3.83±0.50	9.21±0.76	3.07±0.11	4.27±0.03	4.50±0.17	1.16±0.10	6.82±2.44	9.57±0.20
Procyanidin B3	29.45±2.48	22.99±3.85	12.04±1.74	17.35±0.04	24.27±2.34	6.99±1.71	26.14±2.95	30.26±6.4	16.51±3.56
Procyanidin B4	5.61±0.33	5.14±0.62	7.42±2.07	7.35±0.57	8.22±0.87	4.16±1.04	6.42±1.17	7.78±1.92	6.73±1.20
Flavonols *									
Quercetin	0.13±0.04	0.33±0.06	0.28±0.16	0.08±0.04	0.25±0.06	0.34±0.09	0.07±0.00	2.02±0.30	1.03±0.45
Quercetin-3-O-hexoside	2.78±0.49	28.35±5.83	38.07±3.01	3.21±0.65	6.09±2.28	8.56±0.11	2.61±1.03	75.88±1.34	148.13±25.29
Quercetin-3-O-galactoside	0.62±0.08	4.51±1.06	3.99±0.92	1.04±0.10	0.87±0.13	0.75±0.05	0.30±0.04	7.26±0.66	9.66±1.79
Quercetin-3-O-glucuronide	75.14±9.01	154.63±15.09	122.17±6.80	98.93±13.71	44.78±4.22	58.10±21.21	63.90±0.75	227.90±18.01	117.70±12.62
Quercetin-3-O-rhamnoside	0.03±0.02	1.06±0.43	0.45±0.18	0.01±0.00	0.02±0.00	0.02±0.00	0.02±0.00	1.16±0.12	1.10±0.84
Quercetin-3-O-rutinoside	1.33±0.23	1.75±0.11	0.49±0.05	5.46±0.78	3.34±0.28	0.80±0.46	0.38±0.21	0.58±0.50	1.22±1.10
Kaempferol-3-O-glucoside	0.29±0.10	6.78±1.75	4.28±0.10	0.40±0.15	1.67±0.52	0.32±0.12	0.08±0.05	3.87±1.38	9.06±2.37
Stilbenes*									
<i>Cis</i> -resveratrol	0.03±0.02	0.04±0.01	0.14±0.02	0.03±0.01	0.45±0.26	0.09±0.02	0.03±0.00	1.94±0.05	1.43±0.65
<i>Trans</i> -resveratrol	0.11±0.04	0.13±0.02	0.25±0.11	0.04±0.01	0.44±0.38	0.17±0.10	0.13±0.02	4.07±1.21	5.34±0.18
∑ <i>resveratrol</i>	0.15	0.17	0.39	0.07	0.89	0.26	0.16	6.01	6.77
<i>Cis</i> -piceid	0.30±0.06	1.39±0.44	9.63±0.93	1.04±0.48	2.36±1.31	3.00±1.12	0.73±0.21	15.74±6.84	41.99±1.41
<i>Trans</i> -piceid	0.10±0.02	0.36±0.01	1.93±0.37	0.68±0.32	0.52±0.25	1.41±0.28	0.20±0.08	2.12±1.14	7.65±1.53
∑ <i>piceid</i>	0.39	1.75	11.56	1.72	2.88	4.41	0.93	17.86	49.64
<i>Cis</i> -piceatannol	0.03±0.01	0.38±0.19	0.26±0.07	0.05±0.02	0.03±0.00	0.09±0.03	0.03±0.00	9.18±1.52	1.52±0.53
<i>Trans</i> -piceatannol	0.02±0.01	0.23±0.11	1.38±0.80	0.02±0.01	0.02±0.01	0.32±0.15	0.02±0.00	5.48±0.91	10.49±1.93
∑ <i>piceatannol</i>	0.04	0.61	1.64	0.06	0.23	0.41	0.05	14.66	12.01
<i>Cis</i> -astringin	0.03±0.00	0.24±0.00	2.93±0.49	0.04±0.02	0.13±0.08	0.28±0.01	0.04±0.02	0.83±0.33	3.77±0.98
<i>Trans</i> -astringin	0.04±0.01	0.72±0.10	5.91±1.14	0.09±0.06	0.29±0.16	1.70±0.81	0.04±0.02	1.97±0.33	13.27±1.03
∑ <i>astringin</i>	0.07	0.96	8.84	0.13	0.42	1.98	0.08	2.79	17.05
ε-viniferin	0.73±0.19	0.93±0.37	1.61±0.26	0.12±0.04	1.70±0.70	0.63±0.16	0.27±0.11	1.38±0.37	4.77±0.21
ω-viniferin	0.03±0.01	0.04±0.01	0.10±0.04	0.01±0.000	0.07±0.03	0.06±0.01	0.01±0.00	0.27±0.05	0.95±0.04
σ-viniferin	0.01±0.00	0.01±0.00	0.95±0.14	0.01±0.01	0.07±0.02	0.08±0.02	0.01±0.00	0.84±0.13	2.97±0.70
Pallidol	0.02±0.01	0.03±0.01	1.88±1.11	0.03±0.01	0.48±0.30	0.28±0.05	0.05±0.02	1.06±0.46	6.73±2.57
Parthenocissin A	0.06±0.04	0.08±0.07	2.37±1.11	0.06±0.04	0.53±0.37	0.39±0.14	0.05±0.02	1.47±0.18	4.81±0.91
Miyabenol C	0.06±0.01	0.01±0.00	0.22±0.02	0.08±0.06	0.27±0.15	0.16±0.07	0.34±0.02	0.57±0.09	3.05±0.85
Hopeaphenol	0.03±0.01	0.06±0.01	0.26±0.04	0.01±0.00	0.02±0.00	0.03±0.01	0.02±0.00	0.05±0.01	0.22±0.08
Isohopeaphenol	0.03±0.00	0.04±0.01	0.44±0.19	0.04±0.01	0.04±0.00	0.61±0.07	0.03±0.01	1.07±0.31	2.95±0.22

290 *Flavan-3-ols, procyanidins, flavonols and stilbenes are expressed as mg of their corresponding standard per kg of skins FW.
291 Values means \pm SE of 3 biological replicate

292 In order to get more information about the oligomeric and polymeric proanthocyanidins
 293 (also named condensed tannins) the mean degree of polymerization (mDP) of skin
 294 extracts was calculated by means of phloroglucinolysis. It can be noted that mDP
 295 values had a tendency of increase over ripening for Tannat and Syrah skin extracts
 296 (**Table 4**). At M, Syrah showed the highest mDP values very closely followed by Merlot
 297 and Tannat. The same trend has been previously described in Carménère, Merlot,
 298 Cabernet Franc and Cabernet Sauvignon skin extracts at different ripening stages.¹⁰
 299 In addition, it is also worth noting that mDP values in grape skin extracts can vary
 300 substantially. For example, for Cabernet Sauvignon grapes, skin tannin mDP can
 301 range from 3.4 to 85.7.²⁷

302 **Table 4.** Mean degree of polymerization (mDP) values of skins extracts of Merlot,
 303 Tannat and Syrah varieties at different stages of ripening.

Skins	mDP		
	Merlot	Tannat	Syrah
BV	18.05 ± 0.07	13.88 ± 0.48	18.11 ± 0.40
V	32.21 ± 0.94	15.52 ± 0.01	17.62 ± 0.07
M	25.00 ± 0.13	16.08 ± 0.44	25.82 ± 0.90

304

305 *mDP expressed as values means ± SE (*n*=9) of 3 biological replicates × 3 technical
 306 replicates.

307

308 3.2.2. Flavonols

309 Flavonols are important polyphenolic compounds that vary in color from white to yellow
 310 and play an crucial role in the color stabilization (through copigmentation interaction
 311 with anthocyanins) in young red wines. They are also implicated in the sensory
 312 perception of astringency and bitterness.^{28,29} Usually, flavonols are present in berry
 313 skins of both white and colored grapes, and as other grape polyphenols, their content
 314 varies considerably depending on cultivars and ripening stage.²⁵ The same tendency
 315 that for total flavan-3-ols was noticed for flavonols. For Syrah and Merlot cultivars, the
 316 greater values were noted at V (209-332 mg/kg) whereas for Tannat they are observed
 317 at BV (114 mg/kg) (**Figure 1**).

318 As can be expected the individual flavonols identified in skin samples were found in 3-
 319 O-glycoside forms. Among them, quercetin-3-O-glucuronide was the most prevalent
 320 flavonol in all varieties. However, different tendencies can be observed between

321 varieties. Indeed, in Merlot and Syrah cultivars their amounts increased greatly at V
322 stage then diminished at M stage (although the M stage quantities remain more
323 elevated than at BV). On the contrary, the major levels of quercetin-3-O-glucuronide
324 were observed at BV for Tannat skin extracts (**Table 3**). Substantial amounts of
325 quercetin-3-O-glucuronide have also been found in skins extracts of Merlot, Cabernet
326 Sauvignon, Petit Verdot, Syrah, Tempranillo, Garnacha and Garnacha Tintorera.³⁰
327 Quercetin aglycone and other quercetin glycosides (3-O-hexoside, 3-O-galactoside, 3-
328 O-rhamnoside and 3-O-rutinoside) are also present in small quantities in all samples
329 (**Table 3**). In addition to that, kaempferol-3-O-glucoside has also been quantified and
330 their amounts increase during ripening but only for Syrah variety.

331 **3.2.3. Stilbenes**

332 Stilbenes, polyphenolic compounds that belong to the non-flavonoid family, are present
333 in grapes at very low concentrations (1%) and are mainly concentrated in skins.³¹
334 However, due to their importance as bioactive compounds for plant and human health,
335 their characterization on grapes and other foodstuffs is especially interesting.

336 The stilbene content varies significantly versus the ripening stages and the maximal
337 concentrations of stilbenes in grape skin are reached at maturity stage (**Figure 1**). This
338 augmentation is closely related with the expression increase of key enzymes
339 responsible of stilbene synthesis and accumulation: stilbene synthase, phenylalanine
340 ammonia-lyase and 4-coumarate-CoA ligase.³² In accordance with this, our results
341 indicated that the total stilbenes increase at V stage to reach their maximum at M for
342 all studied varieties. Between cultivars, Syrah was the variety richer in stilbenes with
343 107.63 mg/kg skins followed by Merlot (24.11 mg/kg skins) and Tannat (8.17 mg/kg
344 skins) (**Figure 1**).

345 A great variety of stilbenes were identified in all samples (**Table 3**). The authentic
346 standards of several stilbenes (ϵ -viniferin, δ -viniferin, ω -viniferin, partenocissin A,
347 Miyabenol C, hopeaphenol and isohopeaphenol,) isolated and purified in our
348 laboratory were used to identification and quantification purposes. Among them, 4
349 monomers: resveratrol, piceid, piceatannol and astringin (in their *-trans* and *-cis*
350 configurations), 5 dimers: ϵ -viniferin, ω -viniferin, σ -viniferin, pallidol and partenocissin
351 A; one trimer: miyabenol C; and two tetramers: hopeaphenol and isohopeaphenol were
352 identified. Piceid (mainly in the *-cis* form) represents the major compound in all

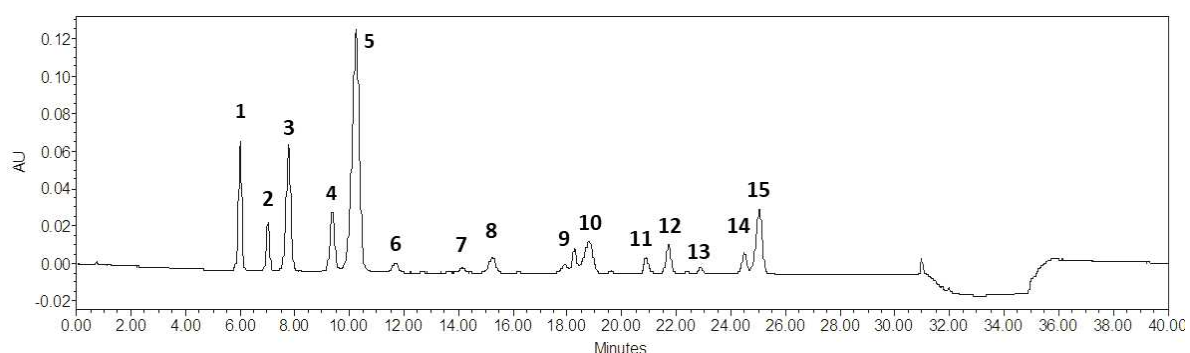
353 varieties (ranging from 0.03-49.64 mg/kg skins) followed by astringin, piceatannol and
354 resveratrol. Important quantities of ϵ -viniferin were also quantified in samples (0.12-
355 4.77 mg/kg skins). Concerning other oligomeric stilbenes, several differences were
356 observed between cultivars. In Merlot, Parthenocissin A was the main oligomeric
357 stilbene but for Syrah it was Pallidol. Very similar results have been recently published
358 in Tannat variety at different ripening stages during 2017 harvest.³³ As far as we know,
359 this is the first time that an identification and quantification of complex stilbenes with
360 authentic standards was carried out in Merlot and Syrah varieties. In addition,
361 partenocissin A and miyabenol C were identified for the first time that in Merlot, Tannat
362 and Syrah cultivars.

363 3.2.3. Anthocyanins

364 Anthocyanins are the most important natural pigments in wine grapes. These
365 compounds are predominately accumulated in skins of red grapes during ripening, and
366 they are also present in the flesh of “teinturier” varieties.³⁴

367 In red grape skins, anthocyanins were the more predominant polyphenolic compounds.
368 In fact, they represented between 84-91% at V and between 94-95% at M in Merlot,
369 Tannat and Syrah varieties (**Figure 1**). Once again, Syrah skin extract was the richest
370 in anthocyanins with values of 7578 mg/kg skins at maturity, followed by Merlot (5707
371 mg/kg skins) and Tannat (4972 mg/kg skins) (**Figure 1**). Fifteen individual
372 anthocyanins, divided in three families (five -3-O-glucosides, five 3-O-acetylglucosides
373 and five-3-O-coumaroylglucosides) were identified in all samples (**Table 5**) (**Figure 2**).

374 **Figure 2.** LC-DAD chromatogram of anthocyanins identified and quantified in samples.



375

376

377 **1:** Delphinidin-3-O-glucoside; **2:** Cyanidin-3-O-glucoside; **3:** Petunidin-3-O-glucoside;
378 **4:** Peonidin-3-O-glucoside; **5:** Malvidin-3-O-glucoside; **6:** Delphinidin-3-O-
379 acetylglucoside; **7:** Cyanidin-3-O-acetylglucoside; **8:** Petunidin-3-O-acetylglucoside; **9:**
380 Peonidin-3-O-acetylglucoside; **10:** Malvidin-3-O-acetylglucoside; **11:** Delphinidin-3-O-
381 coumaroylglucoside; **12:** Cyanidin-3-O-coumaroylglucoside; **13:** Petunidin-3-O-
382 coumaroylglucoside; **14:** Peonidin-3-O-coumaroylglucoside; **15:** Malvidin-3-O-
383 coumaroylglucoside

384

385 It is well established in *V. vinifera* red skin grapes that the 3-O-glucosides are the main
386 forms followed by 3-O-acetylglucosides and 3-O-coumaroylglucosides. Delphinidin,
387 cyanidin, petunidin, peonidin and malvidin-3-O-glucosides were quantified.
388 Additionally, petunidin, peonidin and malvidin-3-O-acetylglucosides and delphinidin,
389 cyanidin, peonidin and malvidin -3-O-coumaroylglucosides were also quantified in all
390 samples. Malvidin-3-O-glucoside was the major anthocyanin followed by peonidin-3-
391 O-glucoside in Merlot skin extracts. However, in the case of Tannat and Syrah,
392 petunidin-3-O-glucoside was the second more predominant anthocyanin. These
393 results were in accordance with other authors that have noticed the same observation
394 in Merlot,¹⁰ Tannat²² and Syrah.²⁶

395

396 **Table 5.** Quantification of individual anthocyanins in skins extracts of Merlot, Tannat and Syrah varieties at different stages of
 397 ripening.

	Merlot			Tannat			Syrah		
	BV	V	M	BV	V	M	BV	V	M
Anthocyanins									
Delphinidin-3-O-glucoside	nd	269.52±18.26	352.42±27.39	nd	145.86±72.87	395.07±134.98	nd	526.08±204.63	396.80±151.81
Cyanidin-3-O-glucoside	nd	265.65±64.55	346.87±26.86	nd	100.56±54.84	156.56±41.43	nd	195.24±105.11	143.69±23.56
Petunidin-3-O-glucoside	nd	86.89±69.48	348.83±17.31	nd	218.86±11.05	624.99±136.52	nd	1090.05±660.16	543.70±184.11
Peonidin-3-O-glucoside	nd	801.77±173.48	1185.78±94.42	nd	176.48±117.3	324.17±33.72	nd	742.67±312.21	709.13±109.47
Malvidin-3-O-glucoside	nd	993.74±238.54	1378.45±69.64	nd	635.40±389.9	1948.62±204.63	nd	2362.31±339.03	2321.65±682.1
\sum 3-O-glucosides	-	2617.58	3612.86	-	1277.18	3449.42	-	4916.36	4114.97
Petunidin-3-O-acetylglucoside	nd	107.48±19.38	125.87±3.82	nd	58.61±24.12	160.74±42.68	nd	185.95±37.20	172.08±34.93
Peonidin-3-O-acetylglucoside	nd	200.81±32.21	300.34±36.02	nd	57.46±20.84	94.67±12.26	nd	279.77±60.58	287.06±18.66
Malvidin-3-O-acetylglucoside	nd	385.37±78.51	572.86±14.73	nd	123.42±67.78	427.06±60.19	nd	785.32±70.90	978.46±203.50
\sum 3-O-acetylglucosides	-	693.66	999.07	-	239.49	682.46	-	1251.04	1437.6
Delphinidin-3-O-coumaroylglucoside	nd	83.91±15.15	134.52±8.02	nd	53.52±20.15	92.14±9.06	nd	151.28±22.97	164.11±8.47
Cyanidin-3-O-coumaroylglucoside	nd	105.93±15.5	126±7.63	nd	66.31±26.66	160.24±24.22	nd	237.91±16.67	226.65±66.26
Peonidin-3-O-coumaroylglucoside	nd	153.37±22.60	250.27±31.35	nd	59.60±24.76	118.29±10.51	nd	444.01±83.32	451.89±70.38
Malvidin-3-O-coumaroylglucoside	nd	342.07±59.97	585.13±48.62	nd	143.01±80.71	470.23±63.55	nd	905.34±126.90	1183.21±385.2
\sum 3-O-coumaroylglucosides	-	685.29	1095.91	-	322.44	840.9	-	1738.19	2025.86

398 *Results are expressed as mg of their corresponding standard per kg of skins FW. Values means \pm SE of 3 biological replicates \times 3
 399 technical replicates

3.3. Anti-inflammatory and antioxidant potential in vitro effects of skins extracts of Merlot, Tannat and Syrah cultivars at different stages of ripening.

The anti-inflammatory potential of skins extracts of Merlot, Tannat and Syrah at BV, V and M was assessed by the intracellular measure of nitric oxide (NO), and reactive oxygen species (ROS) in a macrophage model cell line (RAW 264.7). NO levels are a well-established marker for the inflammatory process. Indeed, after infection, macrophages are stimulated and can secrete NO and several interleukins in order to destroy the infectious agent. While inflammation is *a priori* a beneficial response, when an excessive production of ROS is chronically generated, the consequence is an important damage at cellular level. In fact, NO is one of the major contributors to the formation of ROS.³⁵ Indeed, chronic inflammation and oxidative stress are considered as a major cause of age related diseases and cancer.¹⁵

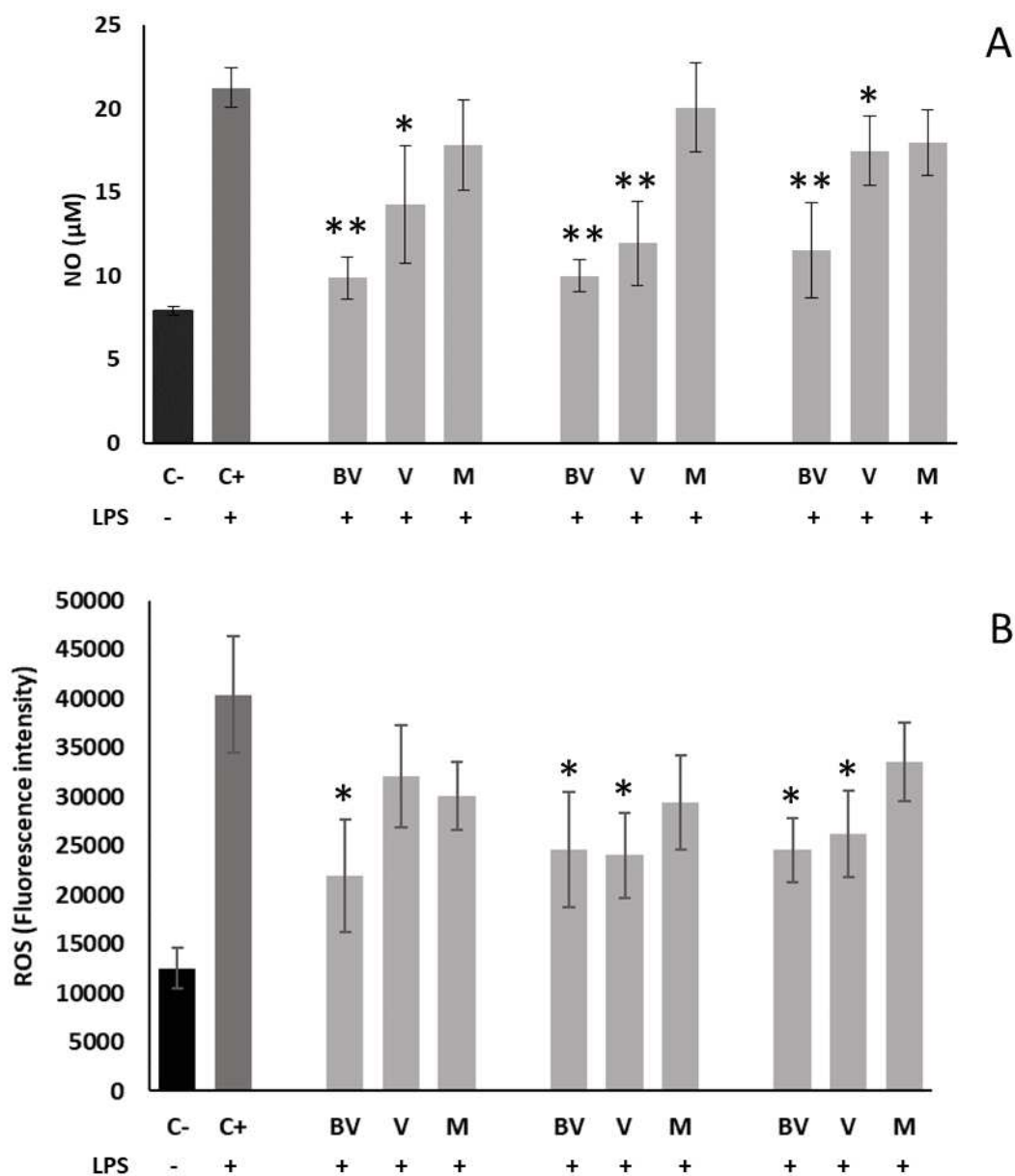
Initially, the cytotoxicity of skin extracts was determined by using the MTT assay, a test based on the reduction of a yellow tetrazolium salt (MTT) to purple formazan crystals by metabolically active cells. For this, cells were treated with different concentrations of skin extracts (50-300 µg/mL) at all experimental conditions (three varieties and three ripening stages) during 24 hours. After analyzing the results, the concentration of 100 µg/mL was selected for further analysis to be non-toxic for the cellular model.

For NO measurement, RAW 264.7 cells were activated with LPS (0.1 µg/mL) in the presence of 100 µg/mL of all skins extracts (three cultivars and three ripening stages). After 24 hours of exposure, culture media were analyzed for nitrite (NO₂) content by the Griess reaction. **Figure 4A** shows the NO production (µM) when cells were treated with LPS alone or with LPS and skin extracts. As can be expected, the NO concentration for positive control (cells treated with LPS) increased threefold (21.2 µM) in comparison with the negative control (7.9 µM) (cells without LPS treatment). When cells were also treated with skin extracts, the NO concentration diminished significantly (around half) for BV and V extracts. In all cases, the most anti-inflammatory sample was the BV extract for all varieties.

In addition, in order to gain insight into the antioxidant potential of skin extracts, the ROS production induced by LPS was investigated by employing the fluorometric probe DCFH₂-DA that is a widely used indicator to detect and quantify the intracellular produced ROS. **Figure 4B** displays the effect exerted by skin extracts after 24 hours

of treatment on ROS production (expressed as fluorescence intensity). As can be clearly observed, the treatment of cells with LPS corresponds to a marked increase of fluorescence (about four times higher) in comparison with negative control proving that the oxidant effect of LPS. However, and in the same way that for NO production, the treatment with LPS + skin extracts produced a significant reduction of ROS production for BV samples of Merlot and for BV and V samples of Tannat and Syrah varieties (**Figure 4B**). Although the individual characterization shows that in general flavan-3-ols, procyanidins, flavonols and stilbenes reached the highest values at V and M stage, it should be taken in consideration that other polyphenolic compounds not quantified in our samples can be the responsible of the observed effects. In fact, TPC values are much higher than the sum of all quantified polyphenols, fact that can explain that BV samples are generally the most anti-inflammatory and antioxidant extracts. Additionally, other bioactive molecules not identified in this work can also contribute to the effect.

Figure 3. NO (μM) and ROS (fluorescence intensity) production in RAW 264.7 cells. Cells were treated for 24 hours by LPS (0.1 $\mu\text{g}/\text{mL}$; + control) or LPS with Merlot, Tannat and Syrah extracts (100 $\mu\text{g}/\text{mL}$) at BV, V and M stages. Results are expressed as mean SEM of four replicates ($n=4$). * p : 0.01-0.05 extracts versus + control, ** p : 0.001-0.01 extract versus + positive.



Among polyphenols, (+)-catechin which is one of the main skin flavan-3-ol at BV stage has shown similar effects in LPS-stimulated RAW 264.7 cells. Thus, (+)-catechin has demonstrated to be able to suppress the NO release by two different pathways, through direct NO scavenging activity and by inhibiting the nitric oxide synthase (iNOS) protein expression.³⁶ In addition, other flavan-3-ol monomers and procyanidins have displayed similar effect in interferon- γ -stimulated macrophages.³⁷ Taking into

account that the major polyphenols in our extracts at V and M stages are the anthocyanins, we can hypothesize that they can be also the responsible molecules of the observed anti-inflammatory and antioxidant effects. This theory can be based on previously results that have demonstrated that malvidin-3-O-glucoside (the major anthocyanin in grape skins) was able to attenuate LPS-induced nuclear factor-KappaB (pro-inflammatory transcription factor associated with NO and interleukin liberation) and ROS production in macrophages.³⁸

In summary, this work provides a wide characterization of individual polyphenolic compounds (41 compounds). This is the first time that an identification and quantification of complex stilbenes with authentic standards have been carried out in Merlot and Syrah varieties. Moreover, parthenocissin A and miyabenol C have been identified in Merlot, Tannat and Syrah cultivars for the first time. Although the major polyphenols content was displayed at BV stage, the HPLC-MS analysis has allowed to monitoring the individual evolution and changes in these compounds during ripening. Whereas anthocyanins, flavonols and stilbenes in general increase during ripening, other compounds such as (+)-catechin (in all cultivars) or quercetin-3-O-glucuronide (in Tannat variety) followed the inverse order. Skin extracts of all varieties at BV stage, have proven to be effective to decrease (>50%) NO and ROS intracellular production in macrophages. More studies should be conducted in order to demonstrate the specific contribution of polyphenolic compounds or other bioactives present in grape skins.

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5. Abbreviations used

BV, before veraison; DCFH₂-DA, 2'7'-dichlorodihydrofluorescein diacetate acetyl; DPPH, 1,1-diphenyl-2-picrylhydrazyl; FW, fresh weight; GAE, gallic acid equivalent; iNOS, oxyde nitrique synthase; M, maturity; MS, mass spectrometry; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide; NO, nitric oxide; ROS,

reactive oxygen species; TPC, total phenolic content; TE, trolox equivalent; UHPLC, ultra-high performance liquid chromatography; V, Veraison

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Chapitre 5

Inhibition de l'enzyme alpha-glucosidase par des extraits de pépins et de pellicules de Tannat au cours de la maturité

L'objectif de ce chapitre était l'étude du pouvoir inhibiteur de l'alpha-glucosidase des extraits de pépins et de pellicules de baies de Tannat (année 2017). L'activité inhibitrice des extraits étudiés a été corrélée d'une part à la teneur en polyphénols totaux, et d'autre part aux teneurs en flavanols, stilbènes et anthocyanes.

Les méthodes utilisées dans ces travaux ont été :

1. une méthode spectrophotométrique pour la détermination du pouvoir inhibiteur des extraits ;
2. une méthode chromatographique couplée à de la spectrométrie de masse pour la détermination des teneurs en flavanols, anthocyanes et stilbènes ;

Les hypothèses étaient :

1. la capacité inhibitrice de l'alpha-glucosidase des extraits de raisin varie entre pépins et pellicules et au cours de la maturité de la baie.
2. les extraits inhibent l'activité de l'enzyme selon un mécanisme d'inhibition compétitif.
3. l'activité inhibitrice est corrélée à la teneur en anthocyanes, flavanols et stilbenes.

Conclusions

1. tous les extraits de baies de Tannat présentent un pouvoir inhibiteur enzymatique contre l'enzyme testée. Pour les extraits de pellicules, l'activité diminue considérablement jusqu'à maturité. Dans les extraits de pépins, elle reste quasi-constante jusqu'à la véraison puis diminue à maturité.
2. les extraits de pépins et de pellicules présentent un mécanisme d'inhibition de type mixte.
3. l'activité inhibitrice des extraits est négativement corrélée avec les stilbènes et les anthocyanes, et positivement avec les flavanols.




Cette étude a fait l'objet d'un article scientifique, publié dans le journal *Biomolécules*, sous la référence :

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Et ci-après présenté.

Article

α -Glucosidase Inhibitory Activity of Tannat Grape Phenolic Extracts in Relation to Their Ripening Stages

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Abstract: The present study aimed to screen grape extracts as novel α -glucosidase inhibitors to prevent type-2 diabetes and hyperglycemia. The total polyphenol content (TPC) was measured by Folin-Ciocalteu assay and the stilbene, anthocyanin and flavan-3-ol compounds were measured by Ultra High-Performance Liquid Chromatography coupled to Mass Spectrometry (UHPLC-MS). The α -glucosidase inhibitory of seed and skin Tannat grape extracts at four ripening stages were investigated. The highest TPC values were measured in seeds at the “veraison stage” (65.29 ± 5.33 g of Gallic Acid Equivalent (GAE) per kilogram of Fresh Weight (FW)). This was in accordance with the high flavan-3-ol contents measured for these two extracts (43.22 ± 2.59 and 45.45 ± 6.48 g/kg of seeds FW, respectively). The skin and seed extracts at the first stage of ripening exerted strong α -glucosidase inhibition, exceeding 95% ($p < 0.05$). A high linear correlation ($R = 0.723$, $p \leq 0.05$) was observed between flavan-3-ol contents and the α -glucosidase inhibitory activity. The stilbene contents and this activity were moderately to strongly anti-correlated ($R = -0.828$, $p \leq 0.05$ for *trans*-resveratrol). The enzyme kinetic studies revealed a mixed type of inhibition. This study brings promising results for the therapeutic potential of seed and skin Tannat grape extracts as a functional food product with anti-diabetic activity.

Keywords: anti-diabetes; grape; ripening; α -glucosidase; phenolic compounds

1. Introduction

Four hundred and sixty-three million people worldwide suffer from diabetes, according to the International Diabetes Federation (IDF) [1]. IDF calls the phenomenon a true pandemic, as the progression is considerable. Predictions suggest that by 2030, 578 million people will have diabetes, and up to 700 million by 2040 [2]. Type-2 diabetes accounts for the majority of diabetes (90%) in the world. Type-2 diabetes, a prevalent endocrine-metabolic disorder, is the result of the body misusing insulin. It is characterized by hyperglycemia which is due to either paucity of insulin secretion by pancreatic β -cells or inefficiency of cells to use insulin against glucose.

Twelve classes of medications, based on different mechanisms of action, are administered as monotherapy or in combination (i.e., metformin, sulphonylureas, glucagon-like peptide 1 analogues, Alpha-Glucosidase Inhibitors (AGI) in type-2 diabetes) [1,3]. Alpha-glucosidase, membrane-bound

intestinal enzyme, is located in the epithelium of the small intestine. It hydrolyzes polysaccharides to *D*-glucose and other monosaccharides, which are then absorbed by the gut, and induces the postprandial hyperglycemia. It was reported that AGIs, oral antihyperglycemic drugs, attenuate postprandial blood glucose [3]. Acarbose is one of the most widely employed drugs for this treatment. It was associated with certain health benefits, such as the diminution in the risk of cardiovascular events. Nevertheless, despite its effectiveness, side effects such as gastrointestinal problems (i.e., diarrhea, nausea, flatulence) can severely limit its use. Therefore, it is interesting to look into the development of a new alternative treatment of natural origin to substitute these chemical compounds administered today.

Polyphenols, which are widely distributed in the plant world, are known for their numerous biological activities, such as antimicrobial, antioxidant, anticancer, anti-inflammatory, cardioprotective activities, or prevention of osteoporosis [4]. Among them, authors have been able to highlight polyphenols, such as stilbenes [5], cyanidin and its glycosides [6], anthocyanins [7,8], tannins [9] or chalcones, hydrocinnamic acids and isoflavones [10] as AGIs in type-2 diabetes pathology.

Grape (*Vitis vinifera* L.) is particularly rich in polyphenolic compounds, such as flavan-3-ols, catechins, anthocyanins and proanthocyanidins [11] (Figure 1). A few studies have demonstrated the inhibitory power of white wine grape pomaces (Chardonnay) against α -glucosidase, thus reducing postprandial hyperglycemia [12–14]. The main research has focused on red grape varieties (i.e., Cabernet Franc, Norton, Chambourcin) [14–17]. Kadouh et al. [16] found major efficiency for Tinta Cão, Syrah and Merlot extracts among six red wine grape pomace varieties on the α -glucosidase inhibitory potential in relation to higher total phenolic content (TPC). At equal or even lower concentrations, all the studied grape extracts demonstrated a superior efficiency compared to the widely prescribed AGI, acarbose. These findings are encouraging results but further investigations are needed to elucidate the complex composition-structure-activity relationships and maybe put on the drug market as a novel AGI. Sun et al. [18] showed, using bio-guided-Thin Layer Chromatography (TLC), that two stereoisomers of 6-*O*-*p*-trans-coumaroyl-*D*-glucopyranoside present in Tinto Cão grape pomace were potential α -glucosidase inhibitors. Other known compounds present in grape were also identified in other food matrices for their anti-postprandial hyperglycemic effect. Epigallocatechin gallate, present in tea extracts, has an important inhibition of α -glucosidase activity [18]. Two *trans*-resveratrol derivatives, rumexoid and piceatannol compounds, also possess some activities [5,19]. Zhang et al. [20] showed that anthocyanidins were more active than anthocyanins in the whole fruits of blueberry, honeysuckle and blackcurrant. The explanation of the anti- α -glucosidase activity by these two families of polyphenols in grape seed was proposed by Yilmazer-Musa et al. [18].

To our knowledge, only one publication was able to highlight the anti-postprandial hyperglycemic effect of skin grape pomaces for Tannat variety grape (origin: Montevideo, Uruguay) [21]. No study investigated the effect of the ripening stage of grapes on the α -glucosidase inhibitory activity of their extracts. The aim of the present study was then:

- To measure the stilbene, anthocyanin and flavan-3-ol content at the different ripening stages.
- To study the α -glucosidase inhibitory activity of seed and skin Tannat grape extracts at four ripening stages.
- To study the type of enzymatic inhibition by determining Lineweaver-Burk plots and kinetics constants calculations.

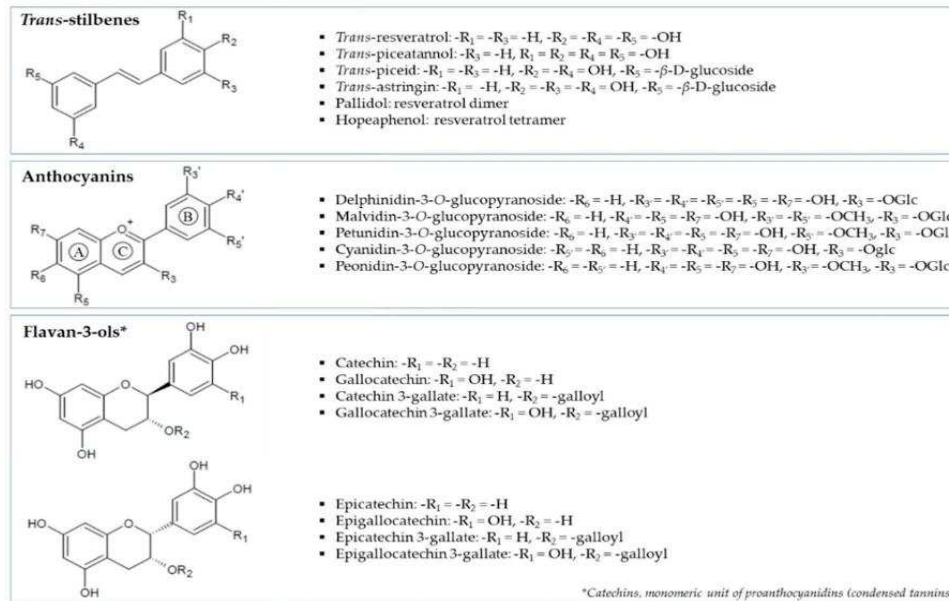


Figure 1. Structures of different polyphenol families and relative compounds identified in seed and skin grapes in the present work.

2. Materials and Methods

2.1. Materials

2.1.1. Chemicals and Reagents

Acetonitrile, sodium carbonate, hydrochloric acid, formic acid, ascorbic acid, gallic acid, Folin-Ciocalteu reagent, catechin, malvidine-3-*O*-glucoside, *trans*-piceid, *trans*-resveratrol, sodium phosphate dibasic dodecahydrate, potassium phosphate monobasic, sodium carbonate and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). Sodium acetate and trifluoroacetic acid were obtained from Carlo Erba Reagents (Peypin, France), and methanol and phloroglucinol from Biosolve Chimie (Dieuze, France). The solvents used were High Performance Liquid Chromatography (HPLC) grade. Deionized water was obtained from a Milli-Q Advantage A10 purification system from Millipore (Fontenay sous Bois, France). Alpha-glucosidase from *Saccharomyces cerevisiae*, acarbose and *para*-nitrophenyl alpha-D-glucopyranoside (*p*-NPG) were purchased from Analytic Lab (Castelnau-le-Lez, France). *Trans*-astringin and *trans*-piceatannol were obtained from Carbosynth (Compton, UK) and ChromaDex (Irvine, CA, USA), respectively. Hopeaphenol, isohopeaphenol, ϵ -viniferin, δ -viniferin and ω -viniferin were isolated from a grapevine raw shoot. The *cis*-isomers were obtained using Ultraviolet-C (UV-C) irradiation (254 nm) from *trans*-isomers.

2.1.2. Fruit Materials

Tannat grapes were harvested at different stages of ripening on the 28 June (first stage), 11 July (before veraison), 25 July (veraison) and 14 September 2017 (maturity) from the INRAe experimental vineyard (Montpellier, France) (Coordinates: 43°37'02.7'' N 3°51'22.3'' E, average annual precipitation: 629 mm, average annual temperature: 15.85 °C, and soil: gravels and river sand). The whole grapes were stored at -80 °C in plastic bags until polyphenol extraction.

2.2. Methods

2.2.1. Polyphenol Extraction

Skins and seeds of thirty Tannat grapes at different stages of ripening were manually separated from the pulp. Biological replicates consist of samples issued from random sampling in the vineyard that were processed separately (three times thirty Tannat berries) to provide a better representation of biological variance across samples for different stages of ripening. A total of ninety samples were collected per ripening stages.

The total polyphenols were extracted from seeds and skins with 100 mL of acetone/water (70/30: *v/v*) then put under deoxygenation with nitrogen for 5 min to minimize the natural oxidation. After stirring for 18 h in the dark, the solution was filtered through a 0.45 μm filter paper then evaporated under vacuum pump V-100 (Suction capacity (1.5 m^3/h , final vacuum (10 mbar)) at 37 °C [22]. The resulting products were freeze-dried between 48 and 72 h under a pressure < 10 Pa and stored at -20 °C until their uses in analytical and enzymatic assays. Skins and seeds were weighted before extraction (Fresh Weight = FW).

2.2.2. Ultra High Performance Liquid Chromatography-Mass Spectrometry (UHPLC-MS) Analysis of Stilbenes

The stilbene analysis was performed by Ultra-High-Performance Liquid Chromatography-Mass Spectrometry (UHPLC-MS) based on a previous method [23]. The freeze-dried skin samples and stilbene standards were solubilized in methanol/water (1/1: *v/v*) at an appropriate concentration (20 g/L).

The system was composed of an UHPLC system (Agilent Technology 1260 Infinity, Agilent Technologies, Santa Clara, CA, United States), hyphenated to an Agilent Technologies 6430 Triple Quadrupole Detector. An Agilent Poroshell 120 EC- C_{18} column (150 \times 2.1 mm, 2.7 μm particle size) was used as a stationary phase. The mobile phases consisted of 0.1% formic acid in water as solvent A and 0.1% formic acid in acetonitrile as solvent B, using the following gradient: 5–17.5% B (0–5 min), 17.5–33% B (5–7.5 min), 33% B (7.5–10 min), 33–40% B (10–15 min), 40–95% B (15–16 min), 95% B (16–19 min) and 5% B (19–21 min). The flow rate was fixed at 0.3 mL/min and the column temperature was set at 35 °C. The injection volume was 4 μL . The detection of stilbenes was in the Multiple Reaction Monitoring (MRM) mode with specific transitions for each compound, and for quantification purposes, a calibration curve was built in the range of 0.05–26 mg/L with pure stilbenes standards. All tests were carried out in triplicate and the results were expressed as milligram per kilogram of skin grapes (FW).

2.2.3. UPLC-Photodiode Array (PDA) Analysis of Anthocyanins

The total anthocyanin content was measured using UPLC-PDA, as described by Pérez-Magariño et al. [24] and Giuffrè et al. [25], with some modifications. The freeze-dried skin samples were solubilized in methanol/water (80/20: *v/v*) at an appropriate concentration.

The UPLC system was a Waters Acquity (Saint-Quentin-en-Yvelines, France), with a photodiode array detector (PDA), LC pump and an auto sampler. The column used was a reversed phase UPLC with an Acquity UPLC BEH C_{18} column (2.1 \times 50 mm, 1.7 μm particle size) (Saint-Quentin-en-Yvelines, France). The temperature of the column was 50 °C. The method used a binary gradient with mobile phases containing 1% *v/v* aqueous trifluoroacetic acid (mobile phase A) and acetonitrile (mobile phase B). The 40 min elution method at flow 0.25 mL/min was 0 min 1% B, 5 min 8.8% B, 30 min 20.6% B, 30.5 min 96% B, 34 min 96% B, 34.1 min 1% B and 40 min 1% B. The detection was monitored at 520 nm. Apparatus was controlled by Empower™ 3 acquisition software (Waters, Saint-Quentin-en-Yvelines, France). A calibration curve of malvidine-3-*O*-glucoside was used. All tests were carried out in triplicate and the results were expressed as gram of Malvidine-3-*O*-Glucoside Equivalent (MGE) per kilogram of skin grapes (FW).

2.2.4. UPLC-PDA Analysis of Flavan-3-ols

Total flavan-3-ols assay using UPLC-PDA was performed as described by Kennedy et al. [26]. Briefly, a solution of 0.1 N HCl in MeOH, containing 50 g/L phloroglucinol and 10 g/L ascorbic acid, was prepared. The freeze-dried skin and seed grapes were dissolved and reacted in this solution at 50 °C for 20 min, and then combined with 5 volumes of 40 mM aqueous sodium acetate to stop the reaction.

The chromatographic apparatus conditions were the same as described in experimental Section 2.2.3. The temperature of the column was set at 40 °C. The method used a binary gradient with mobile phases containing 1% *v/v* aqueous trifluoroacetic acid (mobile phase A) and acetonitrile (mobile phase B). The 20 min elution method at flow 0.45 mL/min was 0 min 2% B, 8 min 6% B, 14 min 20% B, 14.1 min 99% B, 16 min 99% B, 16.1 min 2% B and 20 min 2% B. Eluting peaks were monitored at 280 nm. A calibration curve of catechin was used. All tests were carried out in triplicate and the results were expressed as gram per kilogram of skin or seed grapes (FW).

2.2.5. TPC Determination

The TPC was estimated by the Folin-Ciocalteu colorimetric method [27]. The freeze-dried skin or seed samples were solubilized in methanol (1/200: *w/v*). 20 µL of the diluted extract, 1.58 mL of water, and 100 µL of Folin-Ciocalteu reagent were mixed. After 30 s, 300 µL of 20% sodium carbonate solution (*w/v*) was added. The reaction mixture was thoroughly shaken and left for 2 h in the dark at room temperature (20 °C). Then, the specific absorbance was measured at 700 nm against the blank prepared for each series of determination in such a way that the sample was replaced by the methanol, using an Agilent Cary 60 Ultraviolet-Visible (UV-Vis) spectrophotometer (Santa Clara, CA, United States). The calibration curve of gallic acid was used to determine the concentration of polyphenol in samples. All tests were carried out in triplicate and the results were expressed as gram of Gallic Acid Equivalent (GAE) per kilogram of FW.

2.2.6. α -Glucosidase Inhibition Assays

Set-up of the α -Glucosidase Inhibition Assays in Microplate Format

To test the inhibitory effect of studied skin and seed Tannat grapes harvested at different stages of ripeness, the protocol used was inspired by Pramod et al. [28] based on the use of *p*-NPG, a colorless, unnatural substrate for α -glycosidases. Optimization of reactant volumes, concentrations, pH and incubation times was carried out. The optimized protocol is as follows: 10 µL of freeze-dried grape (Inhibitor) was dissolved in DMSO at 0.4 g/L, then mixed with 60 µL of phosphate buffer solution (100 mM, pH 6.8) containing 10 µL of α -glucosidase enzyme (1 U/mL) into a native polystyrene microwell (Nunc MaxiSorp™, VWR, Fontenay-sous-Bois, France). The Enzyme/Inhibitor (E/I) mixture was subjected to a moderate linear shaking for 15 min at 37 °C in a Heidolph incubator 1000 equipped with a Titramax 1000 shaker (Heidolph Instruments GmbH & Co. KG, Schwabach, Germany). After pre-incubation, 20 µL of a 2.5 mM *p*-NPG (Substrate, S) solution in distilled water was added into each well. The mixture was incubated again for 20 min at 37 °C with shaking. The enzymatic reaction was stopped by adding 50 µL of 0.1 M Na₂CO₃ solution to reach pH 10. At this pH, the resulting hydrolysis product (*para*-nitrophenol, *p*-NP) is under its basic form (yellow color). The 4-nitrophenolate form has a maximum of absorbance at 405 nm. All absorbance measurements were recorded at this wavelength with an Infinite 200™ absorbance microplate reader from Tecan (Lyon, France). This instrument has a measurement range up to 3.6 absorbance units. All experiments were repeated at least four times ($n \geq 4$) per analytical condition. Acarbose, a synthetic AGI, was prepared in distilled water at 0.4 g/L and was used as a positive control for inhibition. Negative control was conducted by replacing the grape extracts with DMSO. No inhibition occurs in that case; thus, maximum absorbance values are obtained with negative controls. Background signal called “blanks” was obtained by substituting the α -glucosidase enzyme with phosphate buffer solution (100 mM, pH 6.8). The blank

value was 0.060 ± 0.004 ($n = 16$). Performance criteria of the optimized protocol (working range, precision and selectivity) were evaluated according to the European Medicine Agency guideline for the validation of bioanalytical methods [29]. Precision of an assay is usually expressed as the standard deviation (SD) or relative standard deviation (RSD) or coefficient of variation CV (the standard deviation divided by the mean value), reported as a percentage. For precision parameters, within-day RSD is noticed as CV_R . Intermediate precision (also called within-laboratory) is expressed as CV_{RW} being carried out by changing operators, conducted on different days, using daily prepared reagent solutions, and different Tannat grape extracts. CV_{RW} includes biological and technical variabilities.

Data Interpretation

All data are reported as the mean \pm the standard deviation (SD) from at least four replicate experiments. The α -glucosidase inhibitory activity was expressed as percentage inhibition by using the following formula:

$$\% \text{ inhibition} = \frac{A(\text{control}) - A(\text{sample})}{A(\text{control})} \times 100 \quad (1)$$

where $A_{(\text{sample})}$ is the measured absorbance at 405 nm obtained with grape extracts and $A_{(\text{control})}$ corresponds to the absorbance of the negative control. Both samples are prepared simultaneously.

Application of the Microplate Assays to Kinetic Studies of α -Glucosidase Inhibition

The inhibition modes of α -glucosidase by skin and seed Tannat grape extracts at the first stage of ripening were determined by using Lineweaver-Burk plots. The Michaelis-Menten constant (K_m) and the maximum reaction velocity (V_{\max}) were determined following the optimized protocol described previously. The experiments were carried out with increasing concentrations of α -glucosidase solution (0.025, 0.050, 0.075 and 0.100 g/L) and *p*-NPG (2.5, 3.0, 3.5, 4.0 and 4.5 mM). The absorbance readings for enzymatic kinetic were started just after the addition of the substrate solution. The released *p*-NP was monitored at 405 nm every minute for 50 min. All absorbance values were included in the working range of the above optimized procedure. All inhibition assays were carried out in triplicate.

2.2.7. Statistical Analysis

The total polyphenolic content and the α -glucosidase inhibitory effects of grape skin or seed extracts were tested by an analysis of variance (ANOVA) on all the data. A Tukey test was carried out and where $p < 0.05$ was considered as significant. Pearson's correlation coefficient (R) and stepwise linear regression were carried out for the determination of correlations between the dependent variable (α -glucosidase inhibitory activity) and the four independent parameters (polyphenolic families, stilbene, anthocyanin and flavan-3-ol contents). The *p*-values less than a confidence level of 95% ($p \leq 0.05$) were considered as statistically significant. All these analyses were performed using XLSTAT software (Addinsoft version 19.01, Paris, France).

3. Results and Discussion

3.1. Evolution of Phenolic Composition in Tannat Grape during Maturation

The total phenolic composition (TPC, stilbenes, flavan-3-ols, anthocyanins) from skin and seed Tannat grape extracts during ripening were measured and are detailed in Tables 1 and 2.

3.1.1. TPC Analysis

The TPC from the freeze-dried skins and seeds at different ripening stages were determined, as described in Table 1. Skin and seed grapes was the major source of polyphenol compounds with an abundance in seeds, and the same finding was noted by Xu et al. [30]. In seeds, the highest TPC

values were attained at the before veraison and veraison, compared with other stages. In skins, the TPC values were found to be higher in the first stage and maturity stage. Obreque-Slier et al. [31] showed the same evolution of TPC compared to our study. The TPC declined from 2.2 to 1.1 mg GAE/g in skins, and it increased from 21.8 to 22.5 mg GAE/g then declined to 16.6 mg GAE/g in seeds. This variability in content might be due to the methods and solvents used to extract the phenolic compounds [32] or the environmental stress, variety, agronomical practices, geographic locations, maturity, irrigation and plant pathogen presence [33,34]. The decline of TPC may be explained by the partial oxidation of polyphenol compounds [35]. The content and the phenolic composition differ considerably between seeds and skins and between the different stages of ripening, which will probably act on the biological activity linked to the inhibition of the enzyme α -glucosidase.

Table 1. Total polyphenolic content of skin and seed Tannat grapes at different ripening stages: First Stage (FS), Before Veraison (BV), Veraison (V) and Maturity (M).

Tannat Extracts		Stilbenes * (mg/kg FW)	Flavan-3-ols ** (g/kg FW)	Anthocyanins ** (g MGE/kg FW)	Total Polyphenols * (g GAE/kg FW)
FS	seeds	nd	35.24 ± 2.56 ^a	nd	48.56 ± 1.74 ^{ab}
	skins	0.81 ± 0.20 ^b	24.13 ± 6.68 ^b	nd	30.29 ± 0.75 ^{bc}
B	seeds	nd	43.22 ± 2.59 ^a	nd	58.44 ± 6.1 ^a
	skins	2.81 ± 1.35 ^{ab}	16.23 ± 4.93 ^b	nd	19.43 ± 1.87 ^c
V	seeds	nd	45.45 ± 6.48 ^a	nd	65.29 ± 5.33 ^a
	skins	1.08 ± 0.08 ^b	4.34 ± 0.47 ^c	1.42 ± 0.27 ^b	10.33 ± 1.04 ^c
M	seeds	nd	37.34 ± 3.36 ^a	nd	47.87 ± 6.48 ^{ab}
	skins	5.18 ± 2.04 ^a	4.63 ± 0.19 ^c	8.70 ± 0.75 ^a	27.07 ± 2.92 ^{bc}

* Values are means of three biological replicates. ** Values are means of three biological replicates x three technical replicates ($n = 9$). nd = not detected. Significant differences between treatments at $p < 0.05$ are noted with letters (a–c), as measured by the Tukey test. MGE: Malvidine-3-O-Glucoside Equivalent; GAE: Gallic Acid Equivalent; FW: Fresh Weight

3.1.2. Total Stilbene Content

Stilbenes were found only in skins with a content that varied with the ripening stages. The total stilbene content increased from the first ripening stage to before veraison. At veraison, the content was about 1.08 ± 0.08 mg/kg of skins, which then increased significantly to 5.18 ± 2.04 mg/kg of skins at maturity. Gil-Muñoz et al. [36] have studied the stilbene content of two grape varieties at harvest and have measured levels at 32.50 and 33.67 mg/kg of skin on grapes harvested in 2014, and 19.03 and 31.43 mg/kg of skin on grapes harvested in 2015 for the Monastrell and Tempranillo varieties, respectively.

As for total stilbenes, the concentrations of most individual stilbene compounds increased during ripening (Table 2). The *trans*-piceid presented the highest content at all ripening stages, similar to other grapes (Monastrell and Tempranillo at harvest) that accumulate resveratrol in their glucosylated form, i.e., *trans*-piceid at 10.29 and 9.34 mg/kg skin, respectively [36]. *Trans*- and *cis*-resveratrol were found at lower levels. Our results are in accordance with Gatto et al. [37], who studied the content of resveratrol in 78 *Vitis vinifera* varieties during ripening and had the same evolution. They found that resveratrol content raised from 0.01 and 0.4 to 0.03 and 1.7 mg of resveratrol per kg of grape in the two lowest producing varieties. The same trend was also observed for other grape varieties, such as Pinot Noir, Merlot or Cabernet Sauvignon [38,39]. Stilbenes' synthesis and accumulation was in direct relation with several biotic and abiotic factors, mainly ultraviolet radiation [40], that may explain the variability on stilbenes content found in the literature.

The accumulation of stilbenes from veraison to maturity was in relation to the increases of stilbene synthase, phenylalanine ammoniolyase and 4-coumarate-CoA ligase expression, responsible for the stilbenes' synthesis and accumulation [37]. The stilbene content is influenced by several factors such as varieties, years and viticultural conditions [36]. The climatic conditions of the region act considerably on the stilbenoid compounds content of grapes; it was already shown that in dry and warm climates, the stilbene content is low, whereas it is higher in regions associated with harsher climates [41].

3.1.3. Total Flavan-3-ol Content

The most abundant phenol compounds found in grapes were the flavan-3-ols with a higher content in seeds compared with skins at all ripening stages (Table 1), which is in accordance with previous results [11,42]. In skins, the highest flavan-3-ol content was measured at the first ripening stage and before veraison, then decreased significantly at veraison and maturity. In seeds, the highest content was before veraison and at veraison. Lorrain et al. [43] studied the phenolic composition of two red grape (skin and seed) varieties, Merlot and Cabernet Sauvignon, from veraison to maturity. A similar evolution was observed, with a difference in the content that could be due to the method to extract polyphenols. According to Adams [44], the decline during ripening may be associated to the natural oxidation of flavan-3-ol compounds.

The contents of extractable proanthocyanidins are reported in Table 2. Their content in Tannat skins were lower than in seeds, in agreement with Obreque-Slier et al. [45]. In skins, only catechin was identified as a terminal unit, while epicatechin-phloroglucinol and epigallocatechin-phloroglucinol were identified as an extension unit with an abundance of epicatechin-phloroglucinol, which is in agreement with Downey et al. [46]. The catechin and epicatechin-phloroglucinol contents decreased significantly from the first stage to veraison. Oppositely, epigallocatechin-phloroglucinol increased and it was absent at veraison and maturity. Obreque-Slier et al. [45] have demonstrated that in skins of both Carménère and Cabernet Sauvignon, catechin was identified as a terminal unit that coincided with our study, whereas epigallocatechin-phloroglucinol, epicatechin-phloroglucinol, catechin-phloroglucinol and epicatechin-3-*O*-gallate-phloroglucinol were identified as extension proanthocyanidin units. The absence of these compounds in our study may be due to a variety effect. In seeds, catechin, epicatechin-phloroglucinol and epicatechin gallate were found as a terminal unit, whereas catechin-phloroglucinol, epicatechin-phloroglucinol and epicatechin-3-*O*-gallate-phloroglucinol were detected as extension units, with an abundance of epicatechin-phloroglucinol content, which is in agreement with Obreque-Slier et al. [45], Downey et al. [46] and Kennedy et al. [47]. The decline of all individual flavan-3-ols after veraison was explained by some partial oxidation phenomenon [47].

3.1.4. Total Anthocyanin Content

The anthocyanins are the colored pigment of skins and were found to be absent in seeds, in accordance with Tkacz et al. [48]. They are also absent in the green stages (first stage and before veraison) of grape skins, in agreement with a previous study [49]. The synthesis started at veraison and accumulated during ripening (Table 1). Our study is in agreement with Boido et al. [50] who studied the total anthocyanins content of Tannat grapes during ripening. They found that the content increased from 1807 mg/kg of grape to 3810 mg/kg of Tannat grapes. The same trend was also observed for other grape varieties, such as Nerello, prunesta, Merlot or Cabernet Sauvignon [25,43].

Nine individual anthocyanins were determined in skin Tannat grapes from veraison to maturity and are reported in Table 2. The content of all individual compounds increased from veraison to maturity with an abundance of malvidin-3-*O*-glucoside, in accordance with Mulero et al. [34]. Lorrain et al. [43] showed that malvidin-3-*O*-glucoside was the major anthocyanin compound in Cabernet Sauvignon (7.39 mg MGE/g of Dry Weight (DW) skin) and Merlot (5.17 mg MGE/g DW skin). The variability observed in the content may be due to the content of primary metabolites, especially sugars, that play a major role in anthocyanins biosynthesis [51] and may also be due to the genetic control of varieties studied [52].

Sugars' accumulation during ripening can play a major role in the synthesis of anthocyanin compounds, it was considered as a substrate for anthocyanins formation and as a regulator in the synthesis [53]. The anthocyanin contents were mostly affected by altitude and environmental factors, such as temperature, that stimulate regulatory genes' expression and varieties. It has been found that the higher temperatures, such as 35 °C, favor the anthocyanin degradation, however, the lower temperatures, around 25 °C, induce the anthocyanins synthesis [54].

Table 2. Quantification of individual polyphenols of skin and seed Tannat grapes at different stages of ripening. All values are the mean of three biological replicates.

Polyphenolic Compounds	Skins				Seeds			
	FS	BV	V	M	FS	BV	V	M
Stilbenes (mg/kg FW)								
<i>Cis</i> -resveratrol	0.007 ± 0.001	0.011 ± 0.007	0.016 ± 0.007	0.101 ± 0.028	-	-	-	-
<i>Trans</i> -resveratrol	0.039 ± 0.011	0.014 ± 0.005	0.051 ± 0.007	0.062 ± 0.011	-	-	-	-
<i>Trans</i> -piceid	0.152 ± 0.033	0.151 ± 0.070	0.221 ± 0.005	0.746 ± 0.119	-	-	-	-
<i>Cis</i> -piceid	0.357 ± 0.056	0.309 ± 0.118	0.344 ± 0.026	1.390 ± 0.479	-	-	-	-
<i>Cis</i> -piceatannol	-	-	0.011 ± 0.003	0.152 ± 0.018	-	-	-	-
<i>Trans</i> -piceatannol	-	-	0.013 ± 0.002	0.163 ± 0.017	-	-	-	-
<i>Trans</i> -astringgin	0.032 ± 0.008	0.044 ± 0.009	0.163 ± 0.020	1.273 ± 0.357	-	-	-	-
<i>Cis</i> -astringgin	0.064 ± 0.036	0.108 ± 0.063	0.103 ± 0.017	0.487 ± 0.166	-	-	-	-
ε-viniferin	0.102 ± 0.039	1.669 ± 0.687	0.128 ± 0.016	0.469 ± 0.038	-	-	-	-
ω-viniferin	0.005 ± 0.001	0.418 ± 0.027	0.001 ± 0.000	0.029 ± 0.007	-	-	-	-
δ-viniferin	0.004 ± 0.000	0.037 ± 0.003	0.006 ± 0.002	0.221 ± 0.011	-	-	-	-
Pallidol	0.003 ± 0.001	0.010 ± 0.009	0.003 ± 0.000	0.042 ± 0.004	-	-	-	-
Hopeaphenol	0.008 ± 0.004	0.011 ± 0.004	0.001 ± 0.000	0.011 ± 0.009	-	-	-	-
Isohopeaphenol	0.040 ± 0.004	0.024 ± 0.016	0.029 ± 0.006	0.061 ± 0.002	-	-	-	-
Anthocyanins (mg MGE/kg FW)								
Delphinidin-3-O-glu	-	-	181.85 ± 24.31	1036.81 ± 76.09	-	-	-	-
Cyanidin-3-O-glu	-	-	134.13 ± 18.29	317.03 ± 18.71	-	-	-	-
Petunidin-3-O-glu	-	-	210.89 ± 29.55	1193.13 ± 75.74	-	-	-	-
Peonidin-3-O-glu	-	-	185.68 ± 26.25	694.38 ± 36.33	-	-	-	-
Malvidin-3-O-glu	-	-	455.83 ± 83.27	3609.86 ± 240.78	-	-	-	-
Peonidin-3-O-acetyl-glu	-	-	50.92 ± 2.70	224.57 ± 14.07	-	-	-	-
Malvidin-3-O-acetyl-glu	-	-	79.91 ± 6.48	778.08 ± 62.78	-	-	-	-
Peonidin-3-O-coum-glu	-	-	58.49 ± 6.81	237.19 ± 16.27	-	-	-	-
Malvidin-3-O-coum-glu	-	-	65.67 ± 8.54	604.70 ± 39.03	-	-	-	-
Flavan-3-ols (mg/kg FW)								
Catechin	1539.40 ± 454.85	731.77 ± 156.40	143.71 ± 34.34	-	1942.38 ± 260.92	4620.67 ± 290.95	5462.36 ± 331.06	2892.28 ± 237.32
Epicatechin	-	-	-	-	730.15 ± 126.63	3024.21 ± 384.69	4944.27 ± 303.71	2899.74 ± 292.72
Epicatechin gallate	-	-	-	-	2777.57 ± 353.07	4438.71 ± 297.00	4796.60 ± 405.72	2642.75 ± 199.44
Catechin P	-	-	-	-	2072.75 ± 178.05	2636.23 ± 149.28	2087.75 ± 156.28	1951.14 ± 297.05
Epicatechin P	16,142.07 ± 518.50	10,257.28 ± 276.46	4203.44 ± 305.93	4625.27 ± 135.65	20,745.92 ± 1206.95	21,957.21 ± 543.21	21,907.22 ± 971.52	21,338.75 ± 1436.20
Epicatechin gallate P	-	-	-	-	6972.59 ± 352.99	6539.24 ± 105.90	6251.56 ± 730.40	5619.76 ± 353.71
Epigallocatechin P	6451.77 ± 260.83	5245.72 ± 647.76	-	-	-	-	-	-

FS = First Stage; BV = Before Veraison; V = Veraison; M = Maturity; glu = glucoside; coum = coumaroyl; P = phloroglucinol; FW = Fresh Weight.

3.2. Optimization and Performances of α -Glucosidase Inhibition Microplate Assays

A microplate-based assay for α -glucosidase activity measurement was developed for rapidly determining inhibition effects of seeds or skins Tannat grapes on α -glucosidase (EC 3.2.1.20) activity. This enzyme hydrolyzes (1 \rightarrow 4)-linked α -D-glucose terminal residue of mono- and poly-saccharides. *p*-NPG, a colorless, unnatural substrate for α -glucosidases, was used as an indicator of enzyme activity since its hydrolysis product is *p*-NP. The absorption spectrum of the basic form of *p*-NP displays a maximum at 405 nm. Reactant concentrations and volumes, pH and incubation times were optimized for control α -glucosidase activity. The whole protocol enables 96 samples to be studied from a single measurement, minimizing operator handling errors. The effect on the α -glucosidase activity of the final content of DMSO (6.5% *v/v*) per well was also checked. The optimized protocol is detailed in the Experimental Section. The assay detection mixture was performed in a final volume per well of 140 μ L, and contained 10 mU of α -glucosidase, 4 μ g of inhibitor (grape extracts) and 50 nmoles of the *p*-NPG substrate. The performances of the proposed optimized protocol were controlled by studying its working range and its precision.

The working range of the assay was determined by the measurement of seed extract (maturity stage) calibration standards, prepared as described in the Experimental Section, within the range 0.025 to 1.00 g/L ($n = 4$ replicates per concentration). This experiment was reiterated over three independent days ($n = 12$ data per concentration level). This gave similar inhibition binding curves over the 3 days (data not shown) with good precision (Table 3). In addition, blank samples revealed that there was no interference during the sample analysis. Maximum absorbance values were obtained with negative controls reported as 0 g/L. In Table 3, we can see this $A_{(\text{control})}$ value corresponded to 2.50 ± 0.10 absorbance, which confirmed that using 10 mU of α -glucosidase permitted to obtain homogeneous results from day to day. This corresponds to the upper limit of the working range, meaning (100%) of the active enzyme. Consequently, the half-maximum absorbance obtained in the experimental working range was 1.25 ± 0.10 . This value corresponded to 50% inhibition of α -glucosidase activity. The working range is limited by a concentration of extracts to be no more than 0.5 g/L. Indeed, as reported in Table 3, at 1.0 g/L of inhibitory extracts, variability of the inter-day assays dramatically increased up to about 46.8%, probably because the reaction medium became hazy due to possible insoluble components at this concentration. Thus, the interaction between grape extract and α -glucosidase was not favorable. Based on these results, all the following studies were done with grape extracts prepared at a concentration less than 0.5 g/L.

Precision of the assay was evaluated with within-day (repeatability) and inter-day (intermediate precision) experiments. The precision parameters ((within day RSD noticed as CV_R) and intermediate precision RSD (CV_{RW})) were estimated for negative control and 3 levels of grape extract (0.25 g/L, 0.50 g/L and 1.00 g/L) of extracts solution added to the wells. Repeatability was evaluated by repeating the assays on the same microplate (8 replicates) during the same day using the same solutions. Statistically identical results ($p > 0.05$) were found for all wells. The effect of random events on the precision of the assay was studied by changing factors (3 days, 3 operators and freshly prepared reagents). ANOVA was used to estimate the precision of the assays and calculations with a minimum of 24 replicates performed. Table 3 gives precision results with CV_R at 3.5% and CV_{RW} at 8.8% for the negative control. For extract samples prepared at a concentration lower than or equal to 0.5 g/L, CV_R are less than 11.5% and CV_{RW} ranged from 12.0% to 26.1%. The results were satisfactory for biological products due to the number of analytical steps (extraction, storage with freeze-drying and thaw laboratory preparation, enzyme). The assay fulfilled intermediate precision. So, to directly estimate percent of inhibition of the α -glucosidase activity after being in contact with Tannat grape extract, the formula (1) was applied. The effects of all seed and skin extracts at all stages of ripening were studied.

Table 3. Summary of the precision results of the seed extract at maturity stage.

Added Concentration of Grape Extracts (g/L)	Abs Value (Mean \pm S _R)	CV _R (%)	S _{RW}	CV _{RW} (%)
0	2.50 \pm 0.10	3.5	0.2	8.8
0.25	1.90 \pm 0.10	6.6	0.2	12.0
0.50	0.90 \pm 0.10	11.5	0.2	26.1
1.00	0.30 \pm 0.10	16.3	0.1	46.8

S_R = repeatability standard deviation; CV_R = repeatability coefficient of variation; S_{RW} = reproducibility standard deviation; CV_{RW} = reproducibility coefficient of variation; Abs = absorbance.

3.3. α -Glucosidase Inhibitory Activity of Grape Extracts

The obtained results, expressed in percentage of α -glucosidase inhibition, are illustrated in Figure 2. Positive control with acarbose prepared at 0.4 g/L was carried out simultaneously. The inhibition percentage for this synthetic AGI was 6.46 ± 0.22 .

At the concentration of 0.4 g/L of dry extract, all the extracts showed an inhibition on the enzymatic activity of α -glucosidase. Except for the skin extract at maturity stage, all these natural extracts proved to be more effective against α -glucosidase compared to the acarbose, a commercial AGI. Overall, the inhibitory effects of skin extracts decrease throughout the different stages of ripening, losing almost all of its activity. As for seed extracts, the inhibitory power remains almost constant during the first three stages of ripening and ends up losing almost 50% of its activity at maturity. The first stage of skin extract and seed extracts, first stage and veraison, exerted the strongest inhibition of α -glucosidase. Both seed and skin extracts, at the same stage of ripening (before veraison), have also demonstrated their efficiency. The inhibitory power of these two extracts is nevertheless statistically different from those reported previously. The seed extract at maturity and skin extract at veraison also exhibited significant activity, like inhibition of α -glucosidase. Finally, the skin extract at maturity showed the least activity compared to the other extracts. Its inhibitory potential was less than the chemical control tested at the same concentration. These two extracts were significantly different.

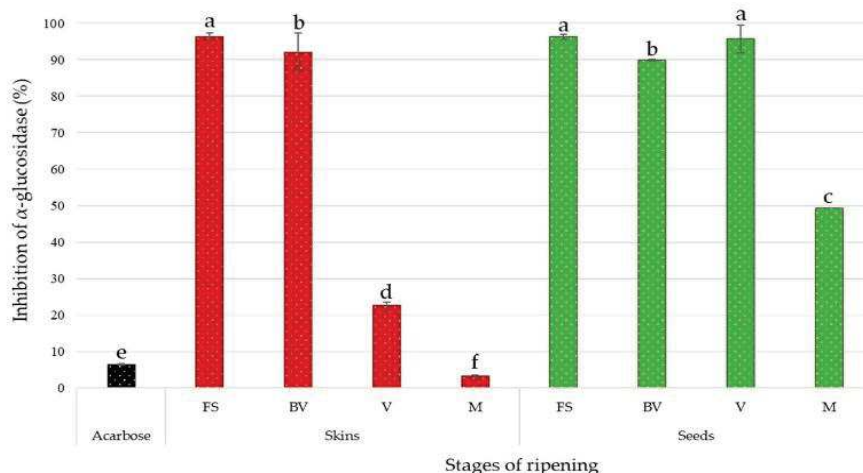


Figure 2. Inhibitory effects of Tannat skin and seed extracts (0.4 g/L) and positive control (acarbose at 0.4 g/L) on α -glucosidase (pH 6.8, T = 37 °C) at different stages of ripening: First Stage (FS), Before Veraison (BV), Veraison (V) and Maturity (M). Values are means of four replicates. Significant differences between treatments at $p < 0.05$ are indicated with letters as measured by Tukey test.

Fernández-Fernández et al. [21] have observed the potential antidiabetic of hydro-alcoholic-acid extract of Tannat grape skin. As opposed to the results obtained in our study, acarbose demonstrated better efficacy than the extracts. For the same variety grape, phenolic composition could be affected by several factors, such as sunlight [54], altitude, climate [54,55], grape ripeness and soil conditions [56]. Nevertheless, the α -glucosidase inhibitory data reported in this manuscript were in accordance with

previous studies. Grape extracts have been shown to be potent inhibitors of the enzyme compared to acarbose [12,14–17]. As reported by Hogan et al. [14], the inhibitory effect (63.9 and 42.4%) of the red and white wine grape pomaces (Cabernet Franc and Chardonnay) at 10 $\mu\text{g/mL}$ surpassed that of acarbose (150 $\mu\text{g/mL}$), which exerted 26.5% inhibition. Also, Zhang et al. [16] showed significant inhibition of red Norton grapes of yeast α -glucosidase at a concentration range of 14.3–285.7 $\mu\text{g/mL}$ compared to commercial AGI (285.7 $\mu\text{g/mL}$). The observed variability of the inhibitory activity may be due to varietal differences and differences in sample preparation conditions.

In order to get further, the mode of action of two of the most active extracts (seed and skin extracts at the first stage of veraison) was investigated. To find the inhibition mechanism against α -glucosidase, inhibitory kinetics were analyzed by Lineweaver-Burk plots and are presented in Figure 3. The reciprocal velocity ($1/v$) versus the increasing substrate concentration was plotted. α -glucosidase presented a Michaelis-Menten constant (K_m) of 0.7395 mM/L for *p*-NPG and a maximum reaction velocity (V_{max}) value of 0.1301 1/min (Table 4). Apparent V_{max} values with the increasing concentrations of seed extracts (0.025, 0.050, 0.075 and 0.100 g/L) decrease, and the K_m values raise. In the presence of increasing concentrations of skin inhibitor, V_{max} values were found to be 0.2657, 0.0823, 0.0283 and 0.0218 1/min and K_m values of 9.0839, 10.0296, 7.0813 and 6.8055 mM/L, respectively. The results revealed mixed inhibition mode for both extracts.

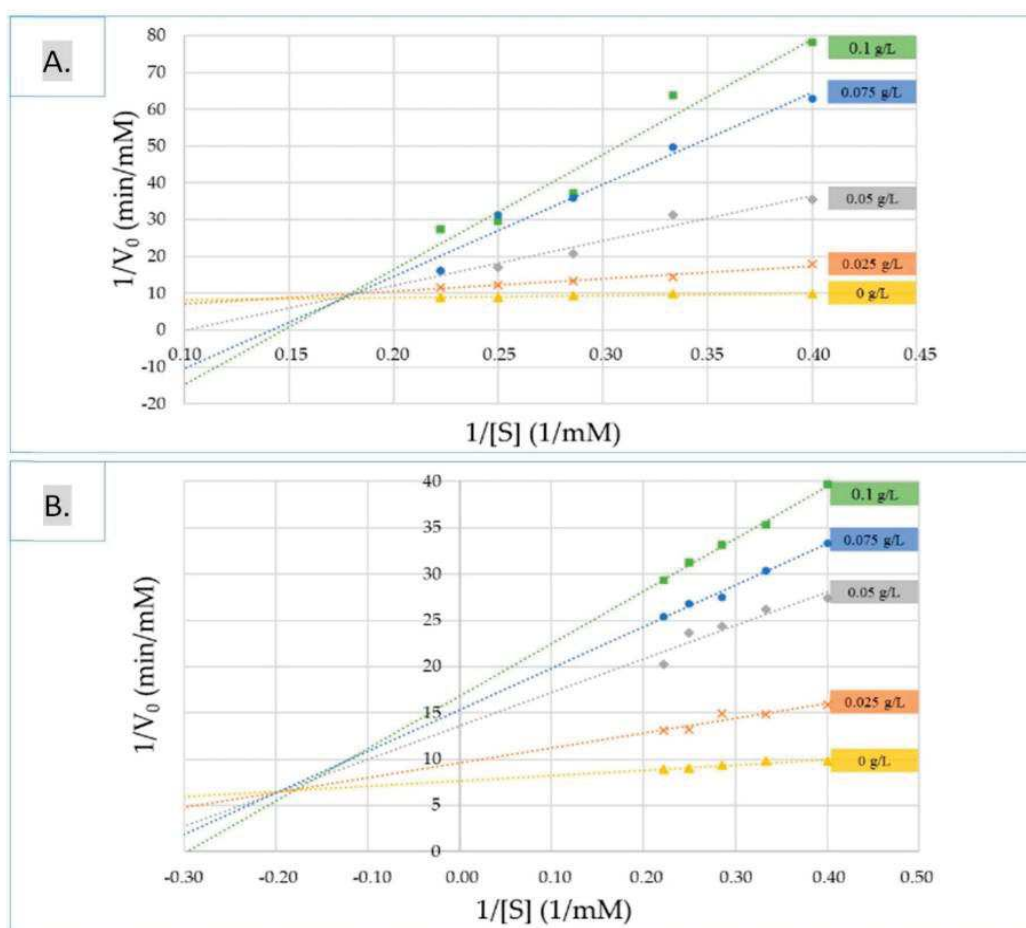


Figure 3. Lineweaver-Burk double reciprocal plots of (A) skin and (B) seed extracts at the first stage of ripening for the α -glucosidase activity mode.

Table 4. Kinetic parameters of α -glucosidase inhibitory capacity by skin and seed extracts at the first stage of ripening.

[I] (mM)	K_m (mM/L)	V_{max} (1/min)
Control		
0	0.7395	0.1301
Skin		
0.025	9.0839	0.2657
0.05	10.0296	0.0823
0.075	7.0813	0.0283
0.1	6.8055	0.0218
Seed		
0.025	1.6743	0.1042
0.05	2.6553	0.0735
0.075	2.9287	0.0653
0.1	3.3618	0.0593

3.4. Correlation between Chemical Composition and α -Glucosidase Inhibitory Activity

Previous results were used to assess the relationship between total polyphenol contents, individual stilbene, flavan-3-ol, anthocyanin compounds and the potential antidiabetic of skin and seed Tannat grape extracts. The Pearson's correlation coefficients (R) and the probability p -values were calculated and are detailed in Figure 4.

A.		Pearson correlation coefficient (R)		p -value
	Stilbenes	-0.623**		0.010
	Flavan-3-ols	0.723*		0.033
	Anthocyanins	-0.781*		0.022
	TPC	0.641 ^{NS}		0.087

B.		Pearson correlation coefficient (R)		p -value
	<i>Cis</i> -resveratrol	-0.675 ^{NS}		0.066
	<i>Trans</i> -resveratrol	-0.828*		0.011
	<i>Trans</i> -piceid	-0.772*		0.025
	<i>Cis</i> -piceid	-0.446*		0.027
	<i>Cis</i> -piceatannol	-0.677 ^{NS}		0.065
	<i>Trans</i> -piceatannol	-0.679 ^{NS}		0.064
	<i>Trans</i> -astringin	-0.762*		0.028
	<i>Cis</i> -astringin	-0.825*		0.012
	ϵ -viniferin	-0.551*		0.016
	ω -viniferin	0.249*		0.015
	δ -viniferin	-0.791*		0.019
	Pallidol	-0.678 ^{NS}		0.065
	Hopeaphenol	-0.648 ^{NS}		0.082
	Isohopeaphenol	-0.684 ^{NS}		0.061

C.		Pearson correlation coefficient (R)		p -value
	Catechin	0.580*		0.013
	Epicatechin	0.345*		0.020
	Epicatechin gallate	0.463*		0.025
	Catechin P	0.286*		0.029
	Epicatechin P	0.724*		0.022
	Epicatechin gallate P	0.434*		0.028
	Epigallocatechin P	0.425*		0.029

D.		Pearson correlation coefficient (R)		p -value
	Delphinidin-3- <i>O</i> -glu	-0.784*		0.021
	Cyanidin-3- <i>O</i> -glu	-0.883**		0.004
	Petunidin-3- <i>O</i> -glu	-0.780*		0.023
	Peonidin-3- <i>O</i> -glu	-0.820*		0.013
	Malvidin-3- <i>O</i> -glu	-0.745*		0.034
	Peonidin-3- <i>O</i> -acetyl-glu	-0.808*		0.015
	Malvidin-3- <i>O</i> -acetyl-glu	-0.736*		0.037
	Peonidin-3- <i>O</i> -coum-glu	-0.805*		0.016
	Malvidin-3- <i>O</i> -coum-glu	-0.739*		0.036

Figure 4. Pearson's correlation coefficient (with $\alpha = 0.05$) between α -glucosidase inhibitory activity of all skin and seed Tannat grape extracts and total polyphenol families (A), stilbene (B), flavan-3-ol (C) and anthocyanin (D) compounds. * Indicates significance at $p \leq 0.05$, ** Indicates significance at $p \leq 0.01$. NS = Non-Significant, TPC = Total Polyphenol Content, glu = glucoside, coum = coumaroyl, P = Phloroglucinol.

In our study, the Pearson correlation test showed that α -glucosidase inhibitory activity was negatively correlated with stilbenes and anthocyanins (Figure 4A). Regarding the TPC, we obtained a positive but non-significant correlation, depending on the desired α -level with the enzymatic inhibition in contrast to flavan-3-ols, which had a significantly positive correlation coefficient.

Additionally, stilbenes were moderately to strongly anti-correlated, with the exception of ω -viniferin, which was weakly positively correlated (Figure 4B). Although Kerem et al. [5] and Zhang et al. [19] demonstrated that *trans*-resveratrol had an inhibitory activity on α -glucosidase, our data did not support this observation. In our study, the skin Tannat extract at the first stage of ripening exhibited a significantly lower content of *trans*-resveratrol (Table 2) with greater inhibitory activity (Figure 2) than the same extract at veraison or maturity stages. The overall contents of stilbenes in skin grape extracts at the first stage and veraison were significantly identical (Table 1). Nevertheless, the latter had shown inhibitory activity with three times less activity. Zhang et al. [19] was able to highlight the common structural characteristic, among 32 stilbenes, presenting the best enzymatic efficacy: the presence of C_{4'}-OH.

The correlation analysis showed that individual anthocyanins were strongly negatively correlated with the potential antidiabetic of grape extracts (Figure 4D). Cyanidin-3-*O*-glucoside presented the strongest negative correlation with a Pearson coefficient of -0.883 ($p \leq 0.01$). These findings suggested that the strong anti-correlation observed for anthocyanins was directly related to the fact that this family was only present in the two samples (skin grapes at veraison and maturity) (Table 1) with the least activity (Figure 2).

The anthocyanin aglycones as well as cyanidin compounds revealed a much stronger antidiabetic activity than its glycoside form, such as cyanidin-3-*O*-galactoside or cyanidin-3,5-diglucoside [6,8,57]. It suggested that anthocyanins were potent inhibitors of intestinal sucrose after being hydrolyzed in intestine. Furthermore, sugar units linked to anthocyanins played an essential role in exerting biological activity, as described by Akkarachiyasit et al. [6]. The replacement of 3-*O*-galactose by 3-*O*-glucose moiety (diastereoisomer/hydroxyl group on C₄-position) of cyanidin revealed less power. The substitution of a disaccharide (rutinose) instead of a monosaccharide of 3-*O*-cyanidin fraction might also be a significant factor in the activity [58]. Sun et al. [17] hypothesized that a hydroxyl substituent at the C₁-position of saccharide moiety in phenolic glycosides, such as the chemical structure of acarbose, would induce the potency of intestinal sucrose inhibition.

Finally, significant positive correlations among all individual flavan-3-ols were observed (Figure 4C). The results indicated that the epicatechin-phloroglucinol and catechin compounds have the strongest correlations, and weak correlation with catechin-phloroglucinol. As a matter of fact, the epicatechin-phloroglucinol content of the two least active extracts was significantly lower than the most active ones (Table 2). The importance of this correlation resides in the fact that this compound has been predominantly identified in all the Tannat grape extracts. Matsui et al. [59] reported that the α -glucosidase inhibitory activity of monomeric flavan-3-ols (catechins) were determined as (-)-epigallocatechin-3-*O*-gallate > (-)-epicatechin-3-*O*-gallate > (-)-epicatechin > (-)-epigallocatechin > catechin. This study also illustrated the importance of stereoisomers of catechins on activity. Catechins, with a 2,3-*trans* structure (catechin-3-*O*-gallate and galocatechin-3-*O*-gallate), showed less activity than epicatechins that have 2,3-*cis* structure ((-)-epicatechin-3-*O*-gallate and (-)-epigallocatechin-3-*O*-gallate). In addition, the galloylated monomers had higher inhibition than non-galloylated. These structural observations of stereoisomerism aspect on α -glucosidase inhibitory activity were also observed by Gamberucci et al. [60].

These observations do not mean that the presence or absence of certain compounds would explain all the activity. Presumptively, the mentioned activities might arise due to synergistic interaction between individual polyphenolic compounds, as described by Brown et al. [61].

4. Conclusions

The present study showed for the first time the evolution of the phenolic composition for seed and skin Tannat grape extracts during grape ripening. It also demonstrated that these extracts have excellent *in vitro* inhibitory potential against α -glucosidase. The anthocyanin and stilbene compounds do not seem to be involved in the inhibition capacity. Flavanols might be involved, as the α -glucosidase inhibition was correlated with their contents, but further research is needed to confirm this hypothesis.

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Conclusion générale

L'objectif global de ce travail était de caractériser la composition phénolique et les activités biologiques (antioxydante, anti-inflammatoire et anti-diabète) d'extraits de pépins et de pellicules de raisin au cours de la maturation. Ces travaux s'inscrivent dans un objectif global de valorisation des extraits obtenus. Pour cela, trois cépages rouges de *Vitis vinifera* (Merlot, Tannat et Syrah) ont été utilisés. Les baies ont été prélevées à différents stades de maturation.

La partie bibliographique a permis de faire une synthèse des connaissances sur l'espèce *Vitis vinifera*, la baie de raisin, les stades de maturation, les familles de polyphénols, la voie de biosynthèse et l'accumulation des composés phénoliques dans les différents tissus (pépins et pellicules) au cours de la maturité.

La deuxième partie de l'étude bibliographique a permis de faire le point sur les activités biologiques des composés phénoliques de la baie de raisin : activités antioxydante, anti-inflammatoire et anti-diabète.

Le deuxième chapitre était une étude bibliographique (revue) sur les stilbènes du raisin et du vin et leur rôle potentiel en tant qu'agents anti-obésité. Les principaux points abordés dans cet article, en cours de révision, ont été :

- La composition en stilbènes des baies de raisin et du vin et ainsi que les méthodes analytiques les plus efficaces utilisées pour leur détection.
- Les effets anti-obésité *in vitro* et *in vivo* connus des stilbènes du raisin et du vin et les mécanismes moléculaires associés.

Les principales conclusions obtenues ont été :

- Plus de 30 stilbènes ont été identifiés dans les raisins et les vins.
- La chromatographie liquide couplée à la spectrométrie de masse est la méthode la plus efficace pour doser les stilbènes.
- Un grand nombre d'études *in vitro* et *in vivo* ont permis de mettre en évidence l'implications positive des stilbènes (spécialement le resvératrol) comme agents anti-obésité en régulant différentes voies du métabolisme des graisses telles que l'adipogenèse, la lipogenèse, la lipolyse et la thermogenèse.

Le troisième chapitre avait pour objectif d'étudier l'évolution de la composition phénolique et de l'activité antioxydante des extraits de pépins et pellicules des trois

cépages rouges Merlot, Tannat et Syrah au cours de la maturité (année 2017). Les principaux résultats obtenus sont résumés ci-dessous :

- Les pépins contiennent plus de polyphénols que les pellicules pour tous les stades de maturité.
- La teneur en polyphénols des pellicules diminue au cours de la maturation, alors que dans les pépins, celle-ci augmente jusqu'au stade proche de la véraison puis diminue.
- Les extraits de pépins ont une activité antioxydante beaucoup plus importante que les pellicules et ce quelle que soit la méthode utilisée.
- L'utilisation de la voltamétrie cyclique nous a permis non seulement de déterminer la capacité antioxydante totale (cas des tests spectrophotométriques) mais aussi de caractériser les composés les plus facilement oxydables qui s'oxydent à des faibles potentiels (charge du premier pic).
- La charge totale (capacité antioxydante totale) et la charge du premier pic des voltammogrammes sont fortement corrélées avec la teneur en flavanols.

Le quatrième chapitre avait pour objectif d'étudier l'évolution de la composition phénolique (flavanols, flavonols, stilbènes et anthocyanes) et des activités antioxydante et anti-inflammatoire des extraits de pellicules des trois cépages rouges Merlot, Tannat et Syrah au cours de la maturité (année 2018). Les principaux résultats obtenus peuvent être résumés ainsi :

- 41 polyphénols ont été quantifiés au sein des échantillons (6 flavanols, 7 flavonols, 16 stilbènes et 12 anthocyanes). Leurs teneurs varient considérablement au cours de la maturation. En général, les teneurs en flavanols diminuent pendant la maturation tandis que celles en anthocyanes et en stilbènes augmentent.
- Tous les extraits de pellicules sont capables de diminuer la production de NO et de ROS dans les cellules, et ce à tous les stades de maturité. Les meilleurs résultats ont été obtenus pour les extraits avant véraison, plus riches en flavanols.

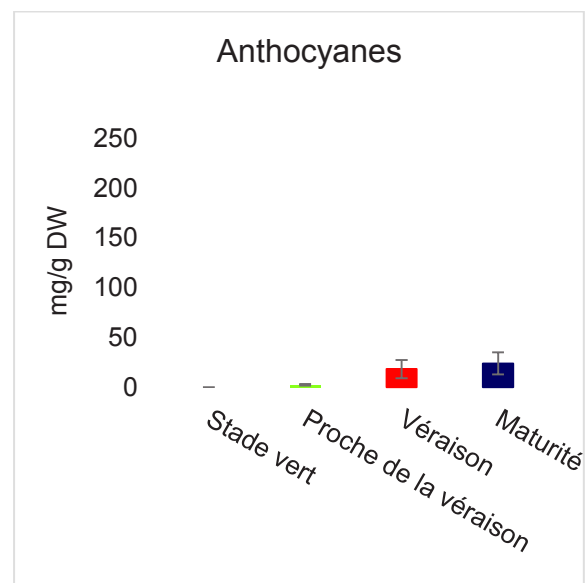
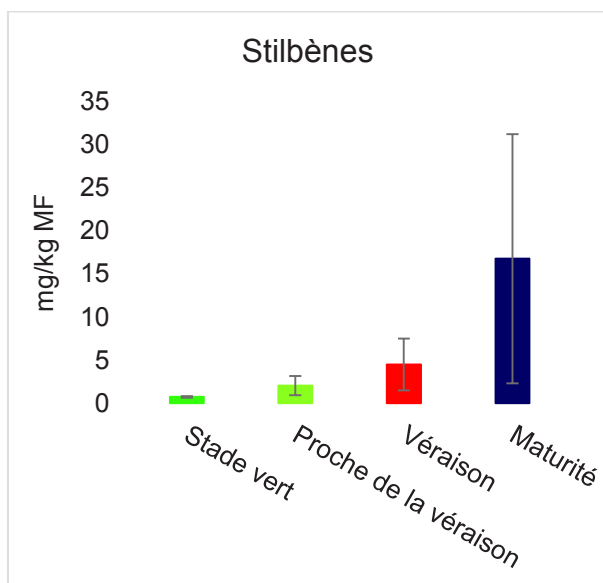
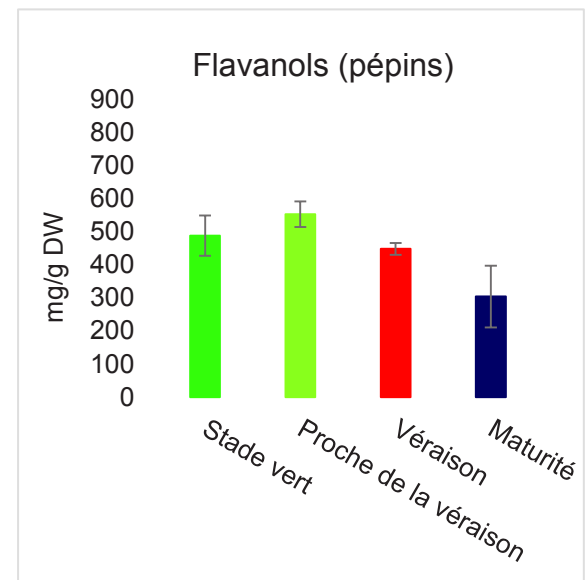
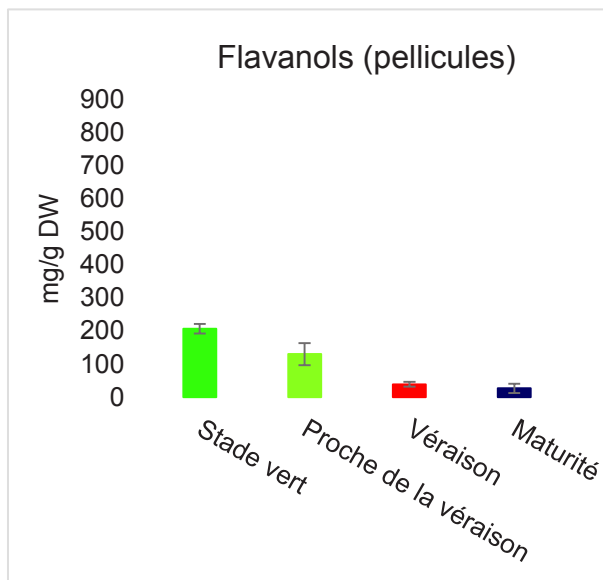
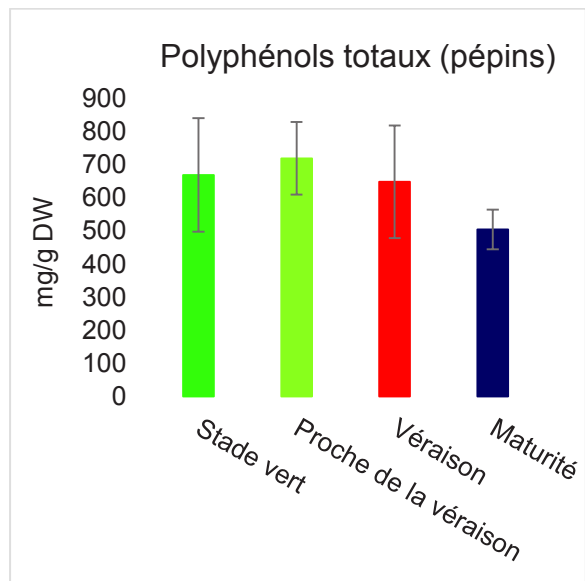
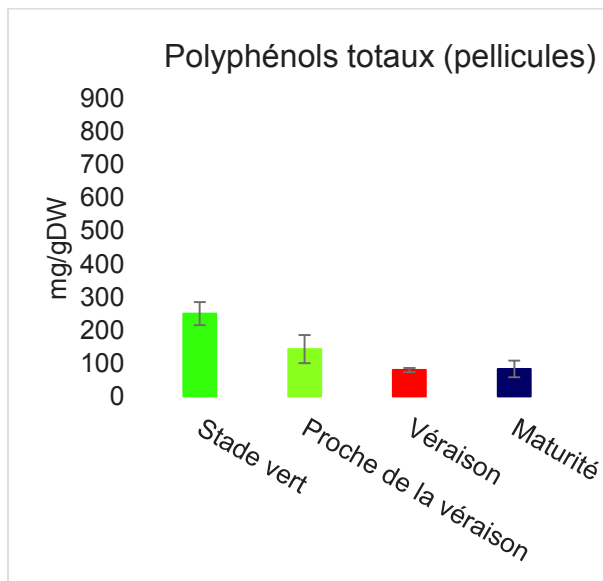
Le cinquième chapitre avait pour objectif d'étudier l'évolution de la composition phénolique (flavanols, anthocyanes et stilbènes) d'extraits de pépins et pellicules du cépage Tannat et de l'inhibition de l'enzyme alpha glucosidase est en lien avec la prévention potentielle du diabète. Il a été montré que :

- La teneur en polyphénols est plus élevée dans les pépins que dans les pellicules pour tous les stades de maturité.
- La teneur en flavanols diminue au cours de la maturation pour les pellicules alors que pour les pépins, celle-ci augmente jusqu'aux stades proche de la véraison et véraison puis diminue jusqu'à la maturité. Les stilbènes et les anthocyanes ont été détectés uniquement dans les pellicules, leurs teneurs augmentant avec les stades de maturation.
- Les extraits de pépins et de pellicules ont une activité inhibitrice beaucoup plus élevée au stade vert par rapport à la maturité.
- Les études cinétiques enzymatiques ont révélé un type mixte d'inhibition de l'enzyme alpha glucosidase par les extraits.
- L'activité inhibitrice est positivement corrélée avec la teneur en flavanols.

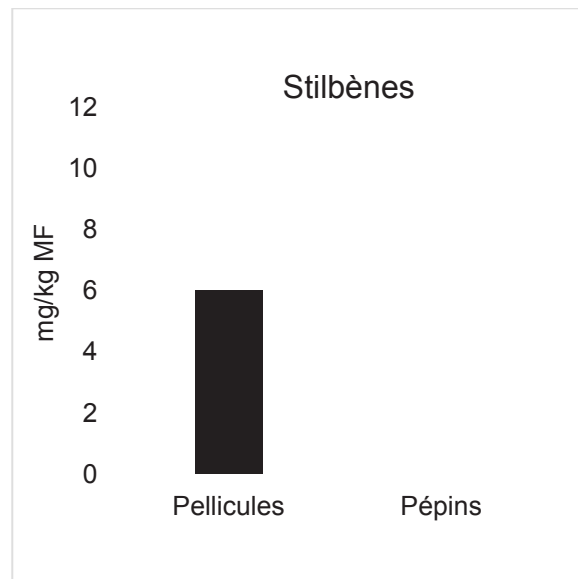
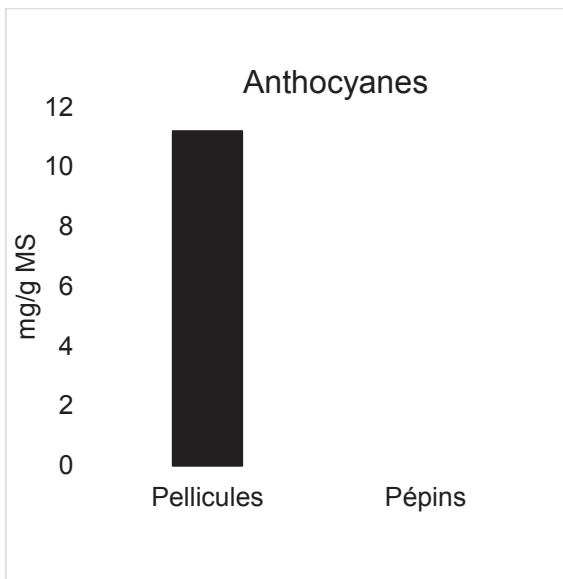
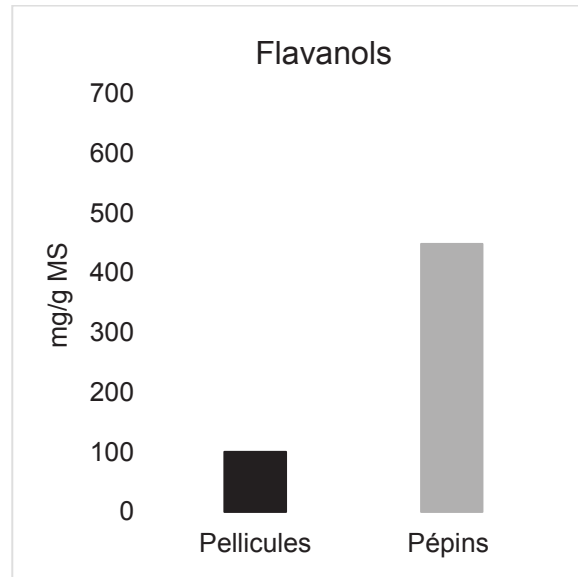
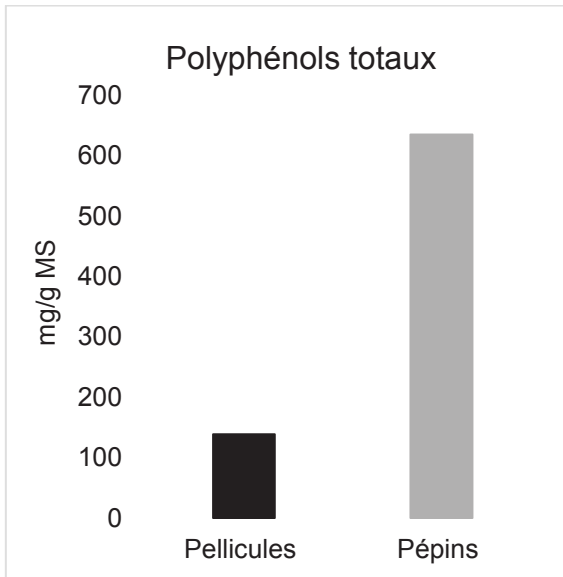
En résumé, nos résultats mettent en évidence l'effet de la maturation, le type de tissu, le cépage et l'année sur l'évolution de la composition phénolique et des trois activités biologiques (antioxydante, anti-inflammatoire et anti-diabète) des extraits de pépins et de pellicules de trois cépages rouges (Merlot, Tannat et Syrah) au cours de la maturation (du stade vert jusqu'à la maturité).

Concernant la composition phénolique

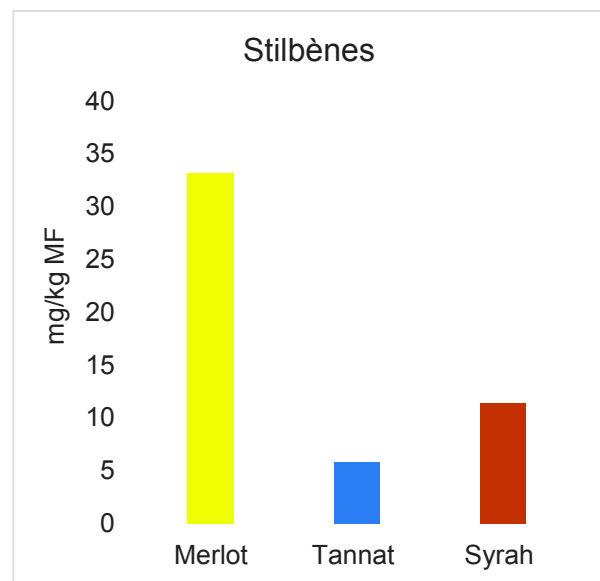
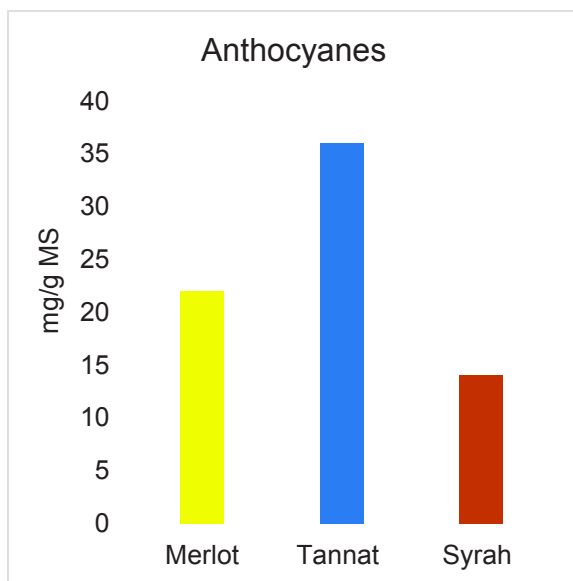
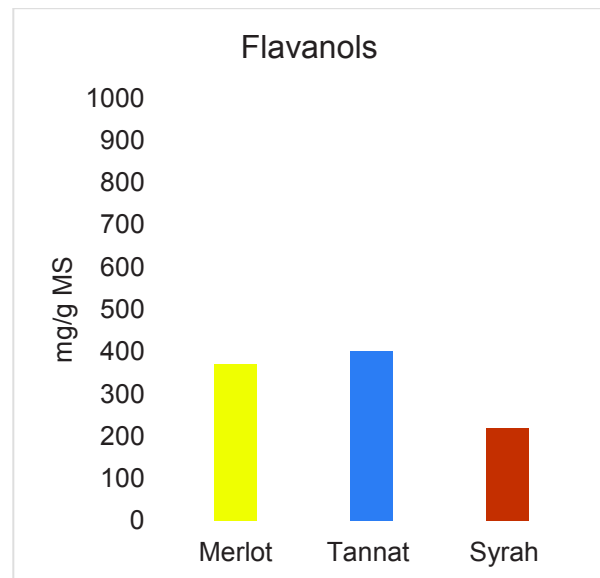
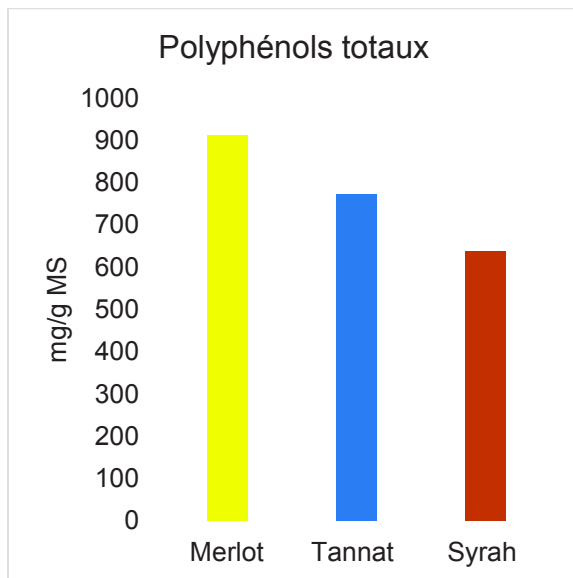
➤ **Effet du stade de maturation** : La teneur en flavanols et polyphénols totaux diminue au cours de la maturation pour les extraits de pellicules et augmente jusqu'au stade proche de la véraison pour les extraits de pépins. Les anthocyanes, qui sont présentes uniquement au niveau des pellicules, leurs synthèses commencent au stade proche de la véraison et s'accumule au cours de la maturation. La teneur en stilbènes augmente au cours de la maturation.



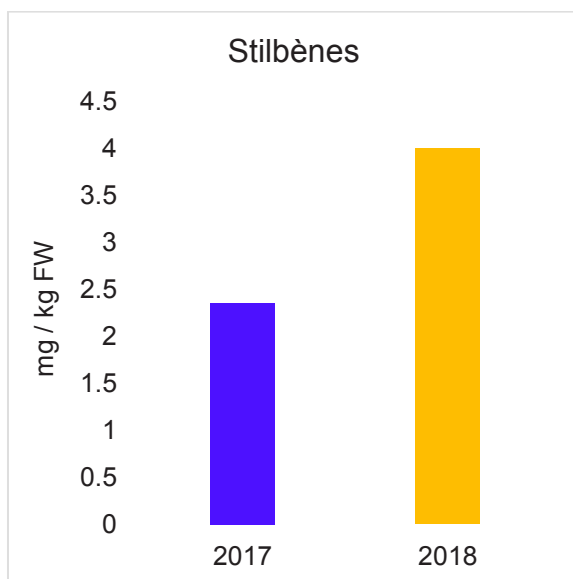
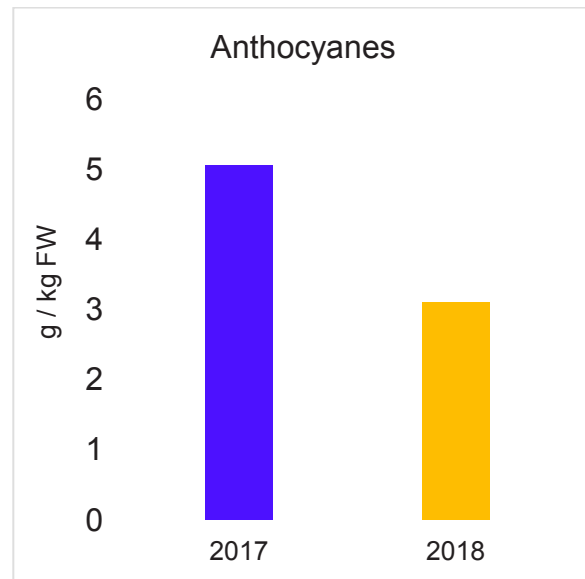
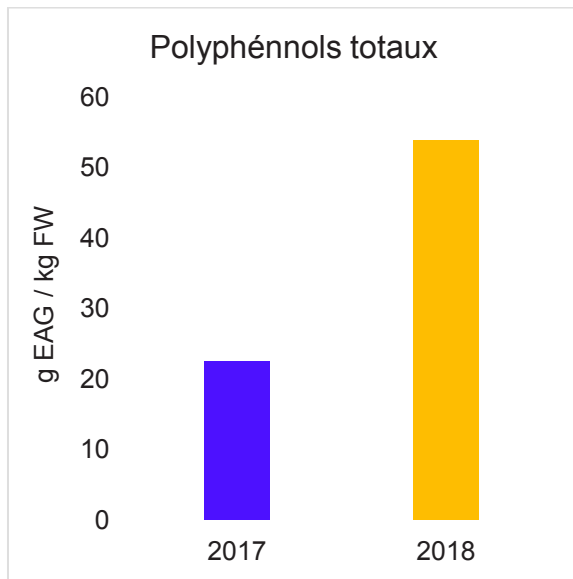
➤ **Effet du tissu** : Les extraits de pépins contiennent plus de polyphénols totaux et de flavanols par rapport aux extraits de pellicules. Les stilbènes et les anthocyanes se trouvent uniquement au niveau des pellicules.



Effet du cépage : Les extraits de Merlot contiennent la teneur la plus élevée en polyphénols totaux et stilbènes, par contre, les extraits de Tannat sont riches en anthocyanes et flavanols.

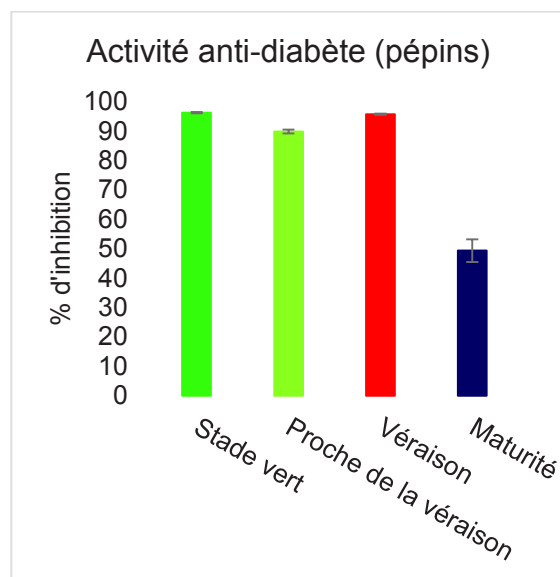
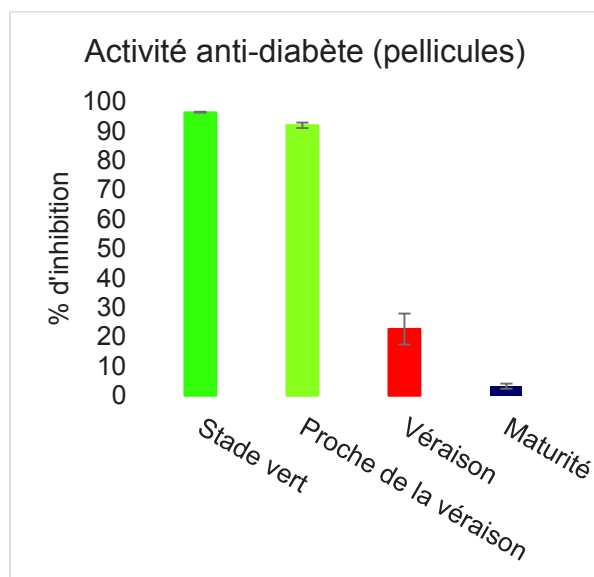
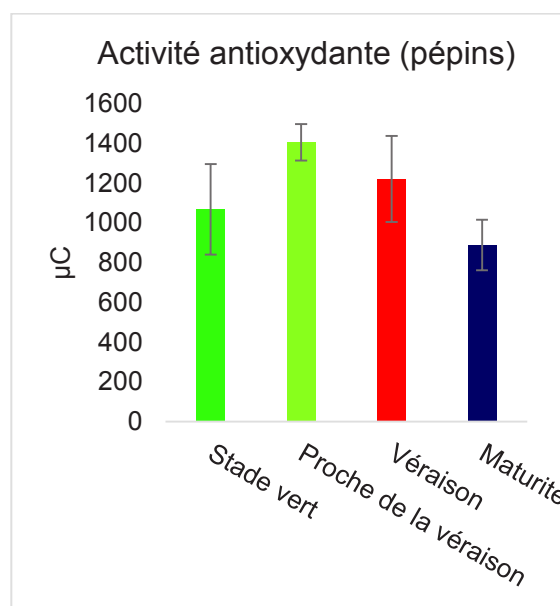
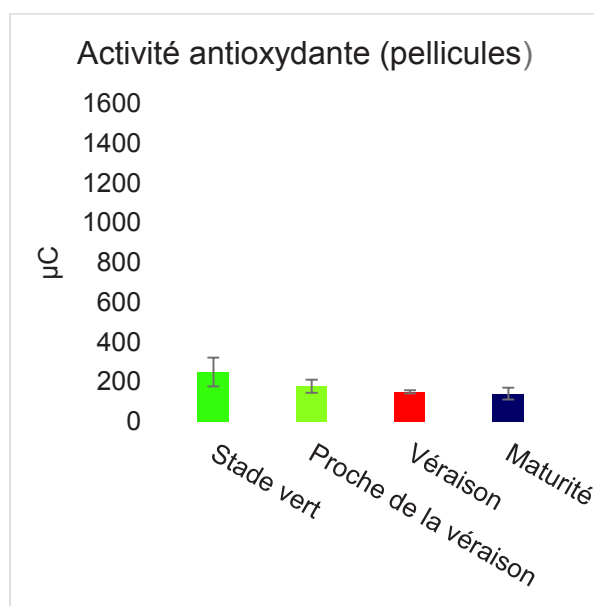


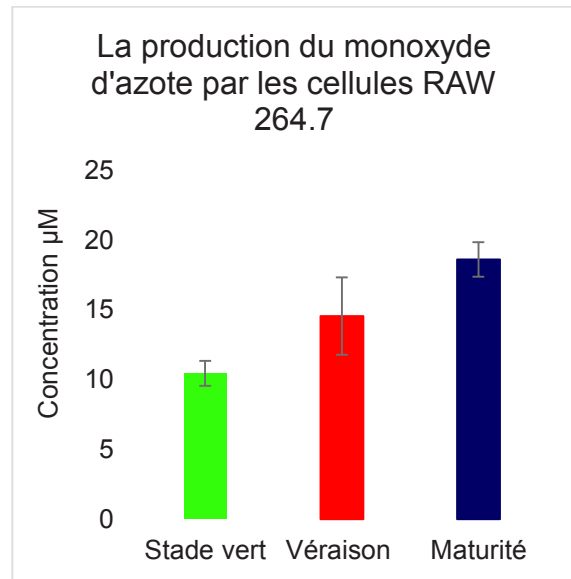
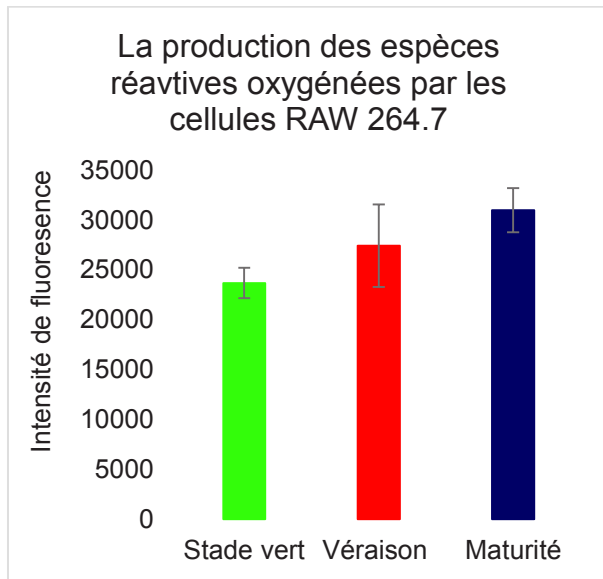
- **Effet de l'année** : Les extraits récoltés en 2018 sont riches en polyphénols totaux et en stilbènes alors que ceux récoltés en 2017 sont riches en anthocyanes.



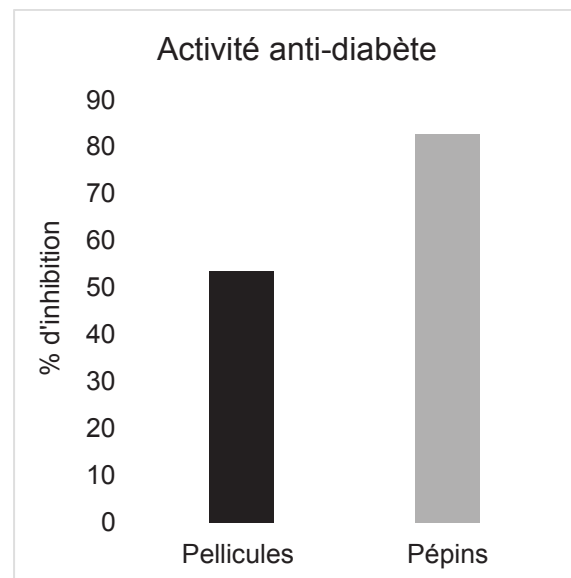
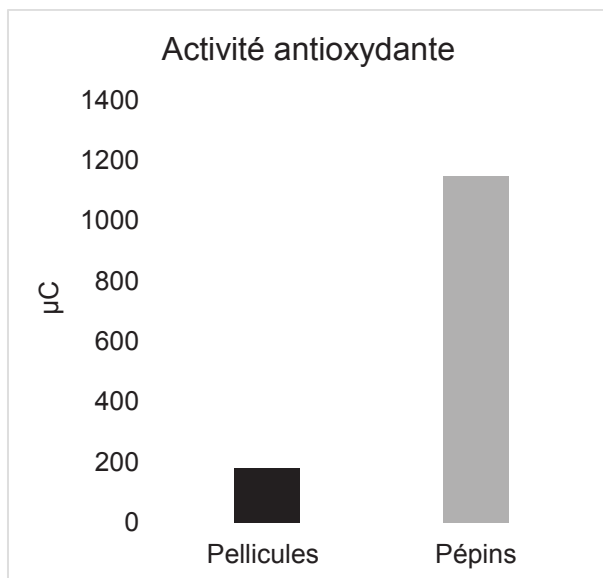
Concernant les activités biologiques

- **Effet du stade de la maturation** : Pour toutes les activités étudiées, le stade vert possède les activités biologiques les plus importantes (dans le cas de l'activité anti-inflammatoire, la diminution de la production des espèces réactives oxygénées et du monoxyde d'azote indique que l'activité anti-inflammatoire est importante).

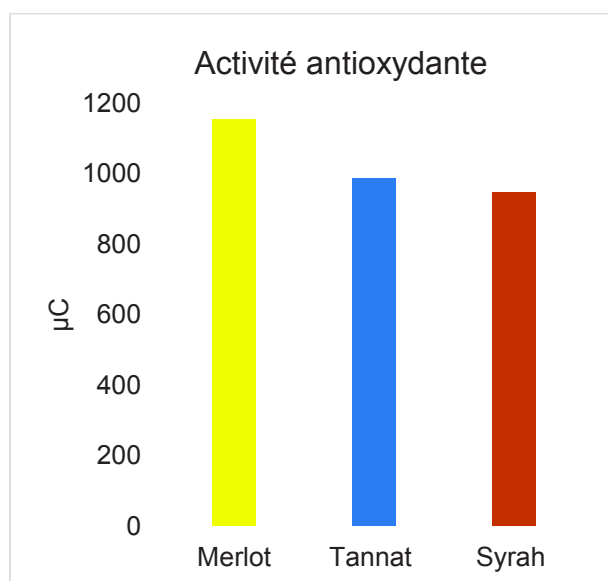
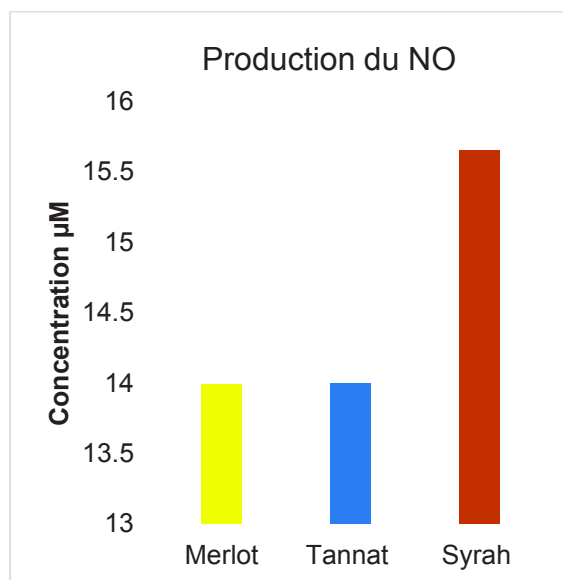
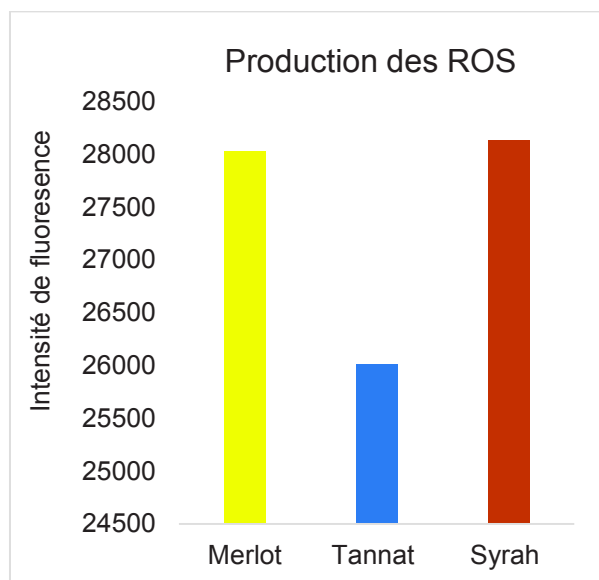




- **Effet du tissu :** Les extraits de pépins possèdent un pouvoir antioxydant et anti-diabétique mieux que les extraits de pellicules.



- **Effet du cépage** : Les extraits de pellicules de Merlot et Tannat possèdent une activité anti-inflammatoire beaucoup plus importante que les extraits de pellicules de Syrah. Dans le cas de la capacité antioxydante, ce sont les extraits de Merlot qui possèdent la puissante activité.



Nos résultats montrent que toutes les activités biologiques étudiées sont plus importantes au stade vert par rapport aux autres stades. Ceci est à rapprocher de la corrélation entre ses activités et les teneurs en flavanols. De plus, une corrélation négative est globalement observée pour les autres familles de composés phénoliques (anthocyanes et stilbènes). On peut donc faire l'hypothèse que les effets observés

pour le stade vert pourraient être dus à certains flavanols présents à la fois dans les pépins et les pellicules comme les proanthocyanidines.

Perspectives

Suite aux résultats obtenus au cours de cette thèse, différentes perspectives peuvent être envisagées. Il serait souhaitable de poursuivre les travaux en se concentrant sur le stade vert qui semble être le plus actif vis-à-vis des activités biologiques pour fabriquer des produits pharmaceutiques, compléments alimentaires ou des médicaments. Afin de poursuivre la compréhension des relations entre structures présentes et activités biologiques des extraits il serait à envisager de :

- tester d'autres cépages ;
- tester différentes méthodes d'extraction des polyphénols en jouant sur le type et la proportion des solvants utilisés ;
- fractionner les extraits à l'aide de gels et de résines pour mieux les caractériser ;
- identifier les composés présents dans les fractions les plus actives ;
- utiliser d'autres tests cellulaires pour confirmer les activités ;
- passer *in vivo* sur les fractions les plus actives.

La poursuite de ces travaux pourrait ainsi ouvrir la voie à des applications dans le domaine des compléments alimentaires ou des médicaments en santé humaine.

Résumé

Les polyphénols appartiennent à la famille des métabolites secondaires présents dans les plantes et majoritairement dans les baies de raisin. Ils jouent un rôle important dans la protection de la plante contre les stress biotiques et abiotiques. Ils ont un impact sur la qualité organoleptique des certains aliments comme ceux provenant du raisin et sont connus majoritairement pour leurs rôles bénéfiques pour la santé humaine. Une étude globale sur la composition phénolique et les activités biologiques (antioxydante, anti-inflammatoire et anti-diabète) d'extraits phénoliques de pépins et pellicules de trois cépages rouges Merlot, Tannat et Syrah à différents stades de maturité a été réalisée durant cette thèse. Les extraits de pépins ont une teneur plus élevée en composés phénoliques que les extraits de pellicules pour tous les stades de maturité. La composition phénolique diffère selon le stade de maturité et le tissu de la baie de raisin (pépin ou pellicule). L'activité antioxydante des extraits a été déterminée par des tests spectrophotométriques (DPPH, ABTS et FRAP) et des paramètres électrochimiques. L'activité anti-inflammatoire a été suivie par l'inhibition de la production des EROs et NO par les cellules RAW 264.7. L'activité anti-diabétique a été déterminée par la mesure d'inhibition de l'enzyme α -glucosidase. Les activités biologiques sont positivement corrélées avec la teneur en flavanols et négativement corrélées avec la teneur en anthocyanes et stilbènes. Les activités biologiques les plus importantes ont été mesurées avant la maturité pour tous les tests et dans les trois cépages étudiés.

Abstract

Polyphenols belong to the family of secondary metabolites found in plants and mainly in grape berries. They play an important role in the protection of the plant against biotic and abiotic stresses. They have an impact on the organoleptic quality of certain foods such as those from grapes and are mainly known for their beneficial roles for human health. A global study on the phenolic composition and the biological activities (antioxidant, anti-inflammatory and anti-diabetes) of the phenolic extracts of the seeds and skins of three red grape varieties Merlot, Tannat and Syrah at different stages of maturation was carried out during this thesis. Seed extracts contain a higher content of phenolic compounds than skin extracts for all stages of ripening. The phenolic composition differs depending on the stage of maturity and the tissue of the grape berry (seed or skin). The antioxidant activity of the extracts was determined by spectrophotometric tests (DPPH, ABTS and FRAP) and electrochemical parameters. Anti-inflammatory activity was followed by the inhibition of ROS and NO production by stimulated RAW 264.7 cells. Anti-diabetic effect was determined by measuring the inhibition of the enzyme α -glucosidase activity. The biological activities are positively correlated with the content of flavanols and negatively correlated with the content of anthocyanins and stilbenes. The most important biological activities were measured before maturity for all the tests and in the three grape varieties studied.