Impact of harvest date on the chemical composition of berries and wines produced from interspecific Vitis sp. cultivars grown in Nova Scotia, Canada over two seasons

Mémoire

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Résumé

La Nouvelle-Écosse présente des conditions climatiques annuelles variables, ce qui en fait un environnement difficile pour la production de raisin pour la vinification. La relation entre la maturité des baies et la composition chimique du vin a été étudiée chez les cultivars de *Vitis vinifera*, mais peu d'études ont porté sur les hybrides interspécifiques *Vitis* sp. comme celles cultivés dans l'Est du Canada. Dans ce contexte, ce projet visait à étudier la relation entre la composition chimique du raisin et du vin, dont les composés volatils libres et liés, chez les hybrides interspécifiques *Vitis* sp. L'Acadie blanc, Osceola Muscat et Seyval blanc récoltés à trois stades de maturation en Nouvelle-Écosse, au cours des saisons 2019 et 2020. Parmi les trois variétés analysées dans cette étude, Osceola Muscat a montré des caractéristiques intéressantes pour la production de vin de climat froid pendant la saison chaude : au dernier stade de maturité (HD3), il a montré un niveau significativement plus élevé de terpènes dans le vin, ce qui suggère que le vin résultant était potentiellement de meilleure qualité, avec des notes florales désirables. Dans des conditions climatiques difficiles, une accumulation plus élevée de GDD (saisons plus chaudes) et une maturité plus tardive (HD3) ont eu un impact positif sur la composition aromatique du vin dans toutes les variétés hybrides interspécifiques de *Vitis* cultivées en Nouvelle-Écosse.

Summary

Nova Scotia shows variable yearly climatic conditions making it a challenging environment for grape production for wine making. The relationship between berry ripeness and wine chemical composition has been studied in *Vitis vinifera* cultivars, but few studies investigated the interspecific hybrid *Vitis sp*. such as those grown in Eastern Canada. In this context, this project aimed at investigating the relationship between the grape and wine chemical composition such as free and bound volatile compounds, in the interspecific hybrid *Vitis* sp. L'Acadie blanc, Osceola Muscat and Seyval blanc harvested at three ripening stages in Nova Scotia during 2019 and 2020. Among the three varieties analyzed in this study, Osceola Muscat showed valuable characteristics for cold-climate wine production in the warmer season at the latest maturity stage, with a significantly higher level of terpenes in wine, suggesting that the resulting wine was potentially of higher quality, with more desirable floral notes. Higher accumulation of GDD (warmer seasons) and later maturity had positively impacted wine aroma composition in all studied interspecific hybrid Vitis varieties in Nova Scotia within challenging climatic conditions.

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List of abbreviations

AC: L'Acadie blanc

AL: Alcohols

ANOVA: Analysis of variance

DMAP: Dimethyl allyl pyrophosphate

DMS: Dimethyl sulfide

FA: Fatty acids

FADP: Fatty acid degradation products

FAEE: Fatty acid ethyl esters

FFP: Frost free period

FW: Fresh weight

GC-MS-FID: Gas chromatography coupled with mass spectrophotometry and flame ionization detector

GDD: Growing degree days

H1, H2 and H3: Harvesting date 1, 2 and 3

HPLC: High performance liquid chromatography

LLE: Liquid–liquid extraction

MT: Mototerpenes

OM: Osceola Muscat

PAN: Primary amino nitrogen

PCA: Principal component analysis

PVPP: Poly vinyl poly pyrrolidone

RDA: Redundancy analysis

SB: Seyval blanc

SBSE: Stir-bar sorptive extraction

SDE: Steam distillation-solvent extraction

SFE: Supercritical fluid extraction

SPE: Solid phase extraction

SPME: Solid phase micro-extraction

TA: Titratable acidity

TSS: Total soluble solids

VP: Volatile phenols and benzene derivatives

YAN: Yeast assimilable nitrogen

Acknowledgements

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Forward

This project aimed at finding the effect of harvest date on the chemical composition of berries and wines produced from interspecific *Vitis sp.* cultivars grown in Nova Scotia, Canada, over two seasons.

The first part consists of a general introduction of the thesis, followed by the first chapter presenting a literature review. The first part of this chapter presents the grape industry and the main grape varieties grown in Nova Scotia. Further, it describes the metabolic changes during berry ripening and abiotic factors affecting berry ripening. Details on the development of wine aroma are explained next. Subsequently, a brief section presents the chemical analyses that can be performed to extract and analyze volatile compounds of grapes and wine. Finally, the last section introduces the hypotheses and objectives of the research project.

The second chapter is presented in the form of a scientific article, of which I am the primary author, supported by Dr. Karine Pedneault, Dr. Martine Dorais, Dr. Paméla Nicolle and Francisco Javier Campos Arguedas. The article is written in English and entitled "Impact of harvest date on the chemical composition of berries and wines produced from interspecific *Vitis* sp. cultivars grown in Nova Scotia, Canada over two seasons".

The implication of each of the authors in this article is as follows: Kumuduni Lakmali: Master's candidate, participation in planning and conducting experiments, analysis of results, and scientific writing of articles. Dr. Karine Pedneault, codirector of master's and principal investigator of the project: project development, student supervision, correction and revision of the manuscript. Dr Martine Dorais, supervisor: collaboration to the project, correction and revision of the manuscript. Dr. Paméla Nicolle: development of winemaking protocol, berry aroma analytical method and manuscript revision. Francisco Javier Campos Arguedas: integration of chromatograms for determination of grape volatile compounds.

Then the last part is a general conclusion, followed by the bibliography references and annexes.

Introduction

Canadian grape production is concentrated mainly in the provinces of Ontario, British Columbia, Quebec, and Nova Scotia. Nova Scotia's commercial wine industry has begun in the early 1980s (Lewis, 2008b). Due to its proximity to the Atlantic Ocean, Nova Scotia shows yearly variations in climatic conditions that can lead to different berry ripeness and quality (Lewis, 2008a). To ensure quality grape production year after year, most NS growers use interspecific hybrid varieties that show higher tolerance to abiotic and biotic stresses (Lewis, 2008b). Hybrid grape varieties are obtained through multiple crosses between native North American species (*V. riparia, V. labrusca, V. rupestris*) and different grape varieties belonging to the species *V. vinifera* (Smiley and Cochran, 2016). Cold climate grape production occurs between last spring and first fall frosts, making cold climate wine quality largely dependent on the climate conditions to properly ripen berries (Pedneault *et al*., 2013). Hence, berries are often harvested earlier than anticipated in cold climate due to quick temperature changes in fall. However, wine quality largely relies on grape ripeness for basic parameters (acidity, alcohol content, etc.) and varietal aroma (Plessis and Rooyen, 2017). Compared to traditional wine grape varieties, hybrid grape varieties may have a higher titratable acidity (Slegers *et al.,* 2015), lower sugar content, and different varietal aromas (Slegers *et al.*, 2017).

Growing-degree days accumulation, which is influences by harvest date, has a significant impact on the chemical composition of grapes, and their varietal characteristics (Pedneault *et al*., 2013). Changes arising during ripening are both physical (volume, weight, color) and chemical (volatile composition, pH, sugars, acidity). Grape ripening is a physiological process initiated with the onset of veraison (Kuhn *et al.,* 2014). The development of berries consists of three main stages: berry development (flower development to bunch closure), the lag phase, and ripening (veraison: berry softening to mature berry) (Flagship and Osmond, 2009). Although several decisive changes occur during the first phase of the development of berries, accumulation of a significant quantity of sugar, anthocyanin and flavour compounds mostly occur during the veraison stage, which affects the quality of the wine (Deloire, 2014; Dokoozlian, 2000).

Because of the importance of grape quality in winemaking, grape maturity has been extensively studied in *V. vinifera* varieties and remains a key topic in viticulture *(Chang et al.*, 2014; Jiang and Sun, 2019; Ubeda *et al.*, 2017). Yet, only a few studies have been done on interspecific *Vitis* varieties (Koziel, 2019; Bowen *et al*., 2016; Slegers *et al.*, 2015; Pedneault *et al*., 2013; Bathe *et al*., 2014).

Therefore, the main objective of this research project was to understand the impact of the ripening stage of berries on the volatile compound profile of grape and wine of the white interspecific hybrid varieties L'Acadie blanc, Osceola Muscat and Seyval blanc produced in Nova Scotia, over two seasons. The physicochemical components (total soluble solids, titratable acidity, pH, etc.) and volatile composition of grapes and wines were measured. Data collected on the volatile composition of berries and wines were used to identify the effect of maturity on the volatile composition of wines and to characterize the aromatic profile of the different grape varieties allowing them to distinguish from each other based on varietal aroma.

1. Literature review

1.1 Overview of grape cultivation in Nova Scotia

1.1.1 Grape industry in Nova Scotia

Canadian grape production is concentrated mainly in the provinces of Ontario, British Columbia, Quebec and Nova Scotia. Nova Scotia's cool climate is marginal for wine grape production in terms of frost-free period (FFP) (>150 days), accumulation of growing degree day (GDD) (>900 degree days above 10 °C), and minimum winter temperatures (rarely below –26 °C) (Lewis, 2008a). The growing season in Nova Scotia has a well-distributed pattern of high rainfall with a mean precipitation of 900 mm a year inland and over 1500 mm a year on the coast with a higher frequency of storms (Province of Nova Scotia, 2021).

1.1.2 Grape varieties

The cultivation of *Vitis vinifera* varieties (e.g., Chardonnay, Riesling and Sauvignon blanc) is complex in Nova Scotia due to its challenging climate. Hence, Nova Scotia's wine industry is based mainly on the production of short-season, cold-hardy French Hybrid varieties, including L' Acadie blanc, Osceola Muscat and Seyval blanc (Jantzi and Mcsweeney, 2019). With appropriate site selection and management, these varieties are among the best performers in Nova Scotia (Lewis, 2008a). These hybrid varieties were developed to combine American species' hardiness and disease resistance attributes with the superior wine quality attributes of the European varieties (Lewis, 2008a).

L'Acadie blanc

L'Acadie blanc is a white Canadian variety issued from a cross of Cascade and Seyve-Villard 14-287. It has good disease resistance and produces wines that tend to be rich and full-bodied, with crisp apple and citrus characteristics (Lewis, 2008a). L'Acadie can withstand temperatures as low as −22 °C to −25 °C, making it much more cold-hardy than the more widely planted Seyval blanc. It is an early to mid-ripening variety that can be very productive and high yielding (Fisher and Jamieson, 2000).

Osceola Muscat

Osceola muscat has a labrusca-like growth habit with good cold hardiness and disease resistance. It tends to be low yielding but produces highly aromatic berries (Lewis, 2008a). It is a mid-season variety in Nova Scotia and produces numerous small to medium-sized grapes (Smiley and Cochran, 2016).

Seyval blanc

Seyval blanc is among the most widely planted hybrids in North America. It is a late mid-season variety with an upright growth habit and low to moderate vigor. It tends to overbear and should be thinned to ensure adequate ripening in a cool climate and maintain vine health (Lewis, 2008a). Seyval produces fresh, light, thin, mineral wines, with dominant apple aromas and slightly spicy notes (Reisch *et al.*, 1979).

A B C

Figure 1.1 Intespecific *Vitis* varieties L'Acadie blanc (A), Osceola Muscat (B) and Seyval blanc (C).

1.1.3 Cultural practices

Besides varietal characteristics, the chemical composition of berries is highly affected by growing conditions, including cultural practices and environmental conditions of the vineyard (climate, soil, microbiota) (Reynolds and Vanden Heuvel, 2009). For instance, optimizing the leaf to fruit balance improves the distribution of photosynthates and promotes the quality and maturity of grapes (Reynolds and Vanden Heuvel, 2009). The choice and maintenance of a suitable training system regulate the vigor of the plants and the leaf area of

the plant cover which affects the interception of light (Jackson *et al*., 1993). Moreover, proper maintenance of the fruit load by removing bunches (cluster thinning) or by disbudding (shoot thinning) (Vos, 2014) can have positive effects on the quality of the fruits by increasing the sugar and pH while lowering titratable acidity (Jackson *et al*., 1993).

1.2 Metabolic changes during ripening

1.2.1 Maturity assessment

The accurate assessment of grape maturity and the determination of the optimal harvesting date are essential for producing quality wine (Du Plessis, 2017). Predicting the optimal harvest date is still difficult, since it depends on the year's climatic conditions year's climatic conditions and environmental factors such as solar radiation, temperature, and water availability (Martinon *et al.*, 2015). In cold climate regions, limited accumulations of heat units during the growing season often led to insufficient ripening of grape varieties, resulting in high fruit acidity levels at harvest. Although the recommended titratable acidity (TA) level for *Vitis vinifera* cultivars at harvest ranges between 6.0 to 10.0 g/L, the TA level of interspecific hybrid cultivars at harvest ranges between 8.0 and 15.5 g/L, with a higher proportion of their acidity coming from malic rather than tartaric acid (Riesterer-loper *et al*., 2019). Maturity assessment of grapes cannot be only measured with total soluble solids (TSS), TA and berry physical appearance (Du Plessis, 2017). Although the sugar content is not at the proper level for harvesting, berries can have a high varietal aroma and flavour, or the opposite (Bremmer, 2010). Difficulties reaching the desired maturity is a particular issue in cold climate grape production, where low sugar content and high TA can happen relatively frequently (Gustafsson and Mårtensson, 2005).

1.2.2 Grape metabolism

Grape quality is mainly determined by their content in primary metabolites such as sugars, organic acids, and amino acids, which evolves with berry ripening (Kuhn *et al.*, 2014). Primary metabolites can be affected by intrinsic factors such as grape variety and ripening stage and extrinsic factors such as growing regions and vineyard management practices (Stefanos 2018). Compared to *Vitis vinifera* varieties, most interspecific varieties show

lower soluble solid content and higher TA values, partly attributable to the short growing season (Pedneault *et al.*, 2013, Khanizadeh *et al.*, 2008).

Sugars

D-glucose and D-fructose are the major sugars present in grapes, but minor quantities of sucrose, D-galactose, D-ribose, D-xylose, D-maltose, D-mannose, trehalose and ribulose 5-phosphate are also found (Eyéghé-Bickong *et al.*, 2012; Coelho *et al.*, 2018). During the initial stages of fruit growth, the sugar concentration of the berry is low, usually around 2% w/w FW. At the onset of veraison, sugar accumulation increases rapidly and reaches more or less 25% w/w FW at harvest (Dokoozlian, 2000) (Table 1.1). Glucose and fructose are present in approximately equal concentrations in grape berries at harvest, ranging from 8 to 12% w/w FW (Dokoozlian, 2000).

Table 1.1 Monosaccharides and disaccharide accumulation (g sugars /100 g FW grape) in *Vitis vinifera* in Thompson seedless berries at different developmental stages.

Sugars	Concentration of sugars (g sugars /100 g berry FW)					
	7 weeks before harvest	6 weeks before harvest	5 weeks before harvest	4 weeks before harvest	3 weeks before harvest	Harvest
Fructose	0.57	3.35	4.88	5.14	7.10	8.05
Glucose	1.87	4.2	5.08	5.37	7.24	8.71
Sucrose	0.09	0.31	0.41	0.46	0.73	0.91
Total	2.52	7.91	10.37	10.97	15.07	17.67

Table modified from Muñoz-Robredo *et al.*, 2011.

Organic acids

Tartaric and malic acids are the primary organic acids in grape berry, making up approximately 90% of total fruit acidity. Minor quantities of citric acid, ascorbic acid, succinic acid, oxalic acid, salicylic acid, cinnamic acid, acetic acid, adipic acid, formic acid, butyric acid and propionic acid have also been identified in grape berries (Shiraishi *et al*., 2010). Several factors such as variety, maturity stage, climate, cultivation area, and year can affect organic acid content in berries (Rusjan *et al*., 2008). As the maturity progresses, and just before fruit ripening, malic acid and tartaric acid content and consequently the total acidity gradually increases in berries. Their concentrations reaches a maximum at veraison

and decline afterwards (Otag *et al.*, 2018). Malic acid is predominantly close to the veraison, followed by tartaric acid, and, three weeks before harvest, tartaric acid accounts for 60 to 80% of total acids (Pavlqušek and Kumšta, 2011) (Table 1.2) (Figure 1.2).

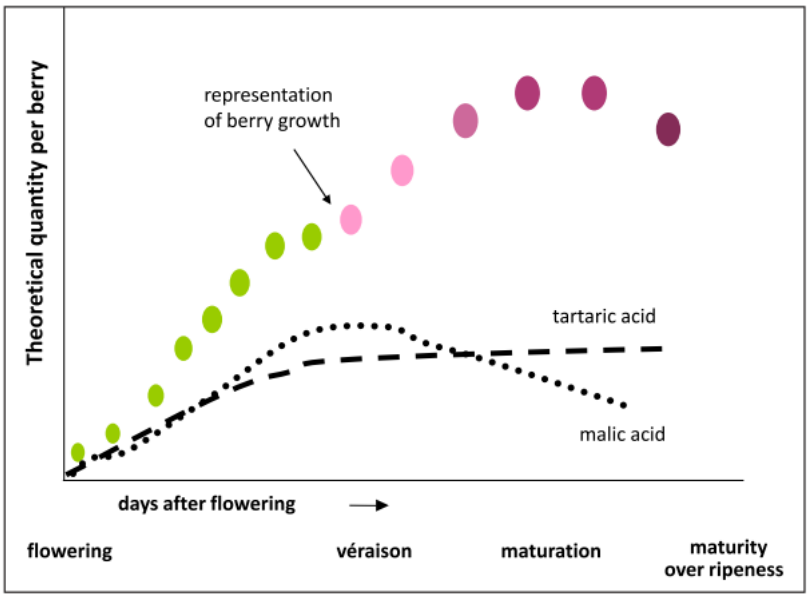


Figure 1.2 Monosaccharides and disaccharide accumulation (g sugars /100 g berry FW) in *Vitis vinifera* in Thompson seedless berries at different developmental stages. (Reproduced from Deloire, 2014)

Table 1.2 Organic acid concentration (g/L of grape juice) in *Vitis vinifera* Thompson seedless berries at different developmental stages.

Organic acids	Amount organic acids (g/L of grape juice)					
	7 weeks before harvest	6 weeks before harvest	5 weeks before harvest	4 weeks before harvest	3 weeks before harvest	Harvest
Tartaric acid	6.55	4.62	2.18	1.54	2.44	2.05
Malic acid	29.92	25.94	10.14	6.29	1.16	1.8
Citric acid	0.4	0.39	0.13	0.03	0.01	ND
Total	36.86	30.94	12.33	7.83	3.6	3.85

Table modified from Muñoz-Robredo *et al.*, 2011.

Temperature is negatively correlated with the concentration of organic acids in berries. In general, fruits ripened in a cooler climate have a higher acidity (mainly due to malic acid) (Chervin et al., 2012) than those ripened in a warm climate.

Grape varietal aroma

The aromatic potential of the grape consists of two groups of compounds offering variable profiles depending on the cultivar:

1) Bound volatile compounds (aroma precursors)
2) Free volatile compounds (terpenols, C_{13}-norisoprenoids, volatile aromatic compounds, etc.) (Deloire, 2014).

The concentration of varietal compounds varies with the grape variety, degree of maturity, vintage, climate, and vineyard management (Flagship and Osmond, 2009). Most varietal aroma compounds are present as non-volatile, odourless, bound forms that enzymatic reactions can release during winemaking and ageing, but can also exist as free molecules in berries (Kuhn *et al.*, 2014).

Analysis of varietal aroma compounds (terpenes, C_{13}-norisoprenoids, and C_6-compounds) can be used as a superior criteria for selecting harvest date (Chang *et al*., 2014). However, some cultivars from the species *V. labrusca* and *V. rotundifolia* have very distinct aromas, as do the *Muscat* varieties, which complexifies this process (Chervin *et al*., 2012).

According to their biosynthetic origin, free volatile compounds in grapes can be classified into six major groups (Di Tomaso, 1996).

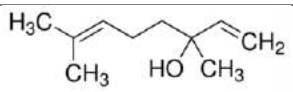
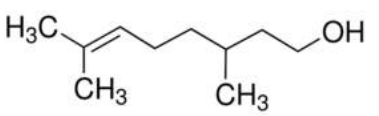
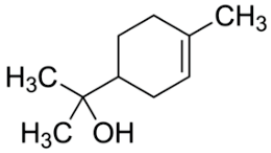
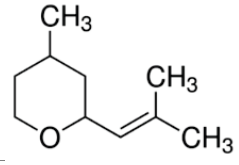
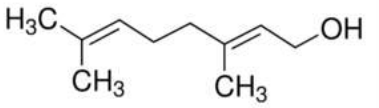
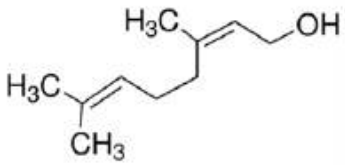
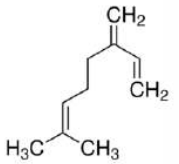
• Terpenoids
• C_{13}-norisoprenoids
• Aromatic compounds
• Aliphatic compounds
• Organo-sulfur compounds
• Methoxypyrazines

Terpenoids

Terpenoids are derived from the universal C_5 precursor, isopentenyl pyrophosphate (IPP), and its allylic isomer dimethyl allyl pyrophosphate (DMAP). Monoterpenes and sesquiterpenes appear to be the most important terpenes with regard to grape aroma composition, and approximately 70 monoterpenes have been identified in grapes and wine (Bohlmann *et al*.,1998). Free monoterpene alcohols that are the most often found in grapes

berries and musts are citronellol, 3,6-dimethyl-1, 5-octadien-1,7-diol, linalool, geraniol, nerol and *α*-terpineol (Table 1.3). Other predominant monoterpenes in grapes and wine include ethers (e.g. rose oxide, nerol oxide) and polyhydroxylated monoterpenes, which are oxidation products from monoterpene alcohols (Dunlevy *et al.*, 2009). Typical aroma descriptions of some important terpenes are floral, rose-like (geraniol, nerol, rose oxides), coriander (linalool), camphoraceous (linalool oxides), green (nerol oxide) and herbaceous (Marias, 1983).

Table 1.3 Structures and aroma descriptors of some terpene key odorants found in grape berries.

Compound	Structure	Aroma descriptors
Linalool		Citrus, orange, floral, terpene, paraffin, rose (1)
Citronellol		Floral, pink, sweet, citrus (2)
α- terpineol		Pine, terpene, lilac, citrus, woody, floral (3)
cis-Rose oxides		Rose, litchi (4)
Geraniol		Citrus, rose (1)(6)
Nerol		Refreshing, citrus, floral, herbaceous (1)
β-myrcene		Earthy, herbaceous, woody, with a hint of rose and shade of carrot and celery (5)

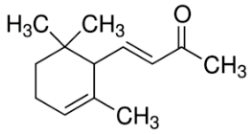
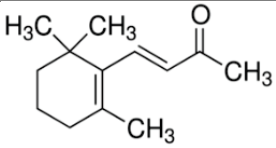
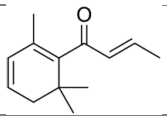
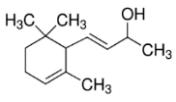
References: [1]Ribéreau-Gayon *et al.*, 1975, [2]Guth 1997, [3]Ferreira *et al.*, 2000, [4]Yamamoto *et al.*, 2002, [5]Buttery *et al.*, 1969 and [6]Hellín *et al.*, 2010.

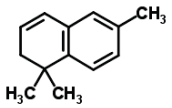
The concentration of terpene volatiles in a berry is affected by many factors, such as grape variety, degree of maturity, and vineyard management techniques. The terpene content varies considerably among cultivars, and cultivars such as '*Muscat*' and '*Riesling*' families are characterized by high terpene content (Di Tomaso, 1996). Even for the same variety, terpene profile can be substantially different in berries from one region to another (Wen *et al.*, 2015).

C_{13}-Norisoprenoids

C_{13}-norisoprenoids derive from the degradation of carotenoids (C_{40}) by chemical, photochemical, and oxidase-coupled mechanisms (Yuan and Qian, 2016). Most research has focused on the C_{13}-norisoprenoids in grapes due to the dominance of norisoprenoids with this number of carbon atoms (Baumes *et al.*, 2002). C_{13}-norisoprenoids accumulate in berries as glycosylated conjugates. These are released by enzymatic or acid hydrolysis during winemaking. Within these volatile, only a few are of importance to wine flavour, including *β*-damascenone, *β*-ionone, 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) and *E*-1-(2,3,6-trimethylphenyl)-buta-1,3-diene (Dunlevy *et al.*, 2009) (Table 1.4). C_{13}-norisoprenoids play an important role in the volatile components of non-floral grapes such as Cabernet Sauvignon, Syrah, Sauvignon Blanc and Pinot noir (Baumes *et al.*, 2002).

Table 1.4 Structures and aroma descriptors of some key *C_{13}*-norisoprenoids odorants found in grape berries.

Compound	Structure	Aroma descriptors
α- ionone		Sweet, woody, floral, violet (1)
β- ionone		Violet, woody (2)
β- damascenone		Plum, cooked apple (2)
α- ionol		Berries, sweet with a floral note and wooded (2)

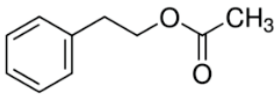
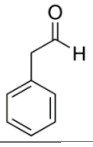
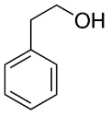
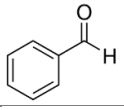
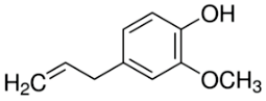
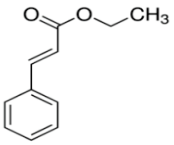
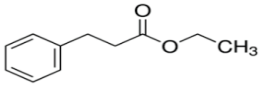
1,1,6-Trimethyl - 1,2 dihydronaphthalene (TDN)		Kerosene-like odour (2)

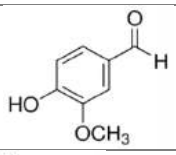
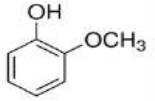
References: [1]Ferreira *et al*., 2000 and [2]Ferreira and Lopez, 2019.

Volatile aromatic compounds

The biosynthesis of most volatile aromatic plant odour compounds is thought to be derived from the shikimic acid pathway (Pichersky *et al*., 2006). Volatiles in this family of molecules significantly contribute to the floral aroma, such as petunia, snapdragon and rose floral scents, and some may also work as plant defense molecules (Dunlevy *et al*., 2009) (Table 1.5).

Table 1.5 Structures and aroma descriptors of some key volatile aromatic compounds found in grape berries.

Compound	Structure	Aroma descriptors
2- Phenylethyl acetate		Sweet, honey, floral (pink) and a raspberry-like (1)
2-Phenylacetaldehyde		Honey-like, sweet, rose, green, grassy (2)
2-Phenylethanol		Sweet, floral, with a hint of rose and honey (2)
Benzaldehyde		Almond, fruity, powdery, nuts (3)
Eugenol		Sweet, spicy (cloves), woody, hints of ham and bacon, cinnamon nuances (2)
Ethylcinnamate		Sweet, balsamic, spices, cinnamon, fruity and powdery (2)
Ethyl hydrocinnamate		Hyacinth, rose, honey, fruity, rummy (2)

Vanillin		Vanilla sugar, creamy, phenolic (2)
Guaiacol		Phenolic, smoked, spicy, drug, vanilla, meat, woody, powdery (4)

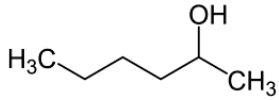
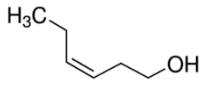
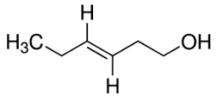
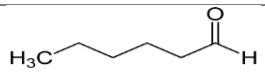
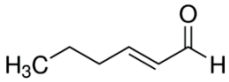
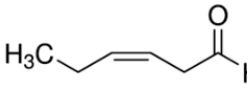
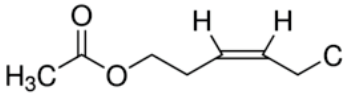
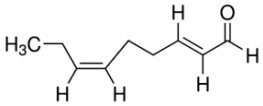
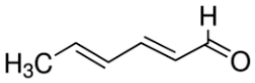
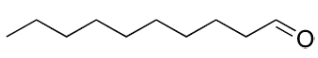
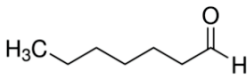
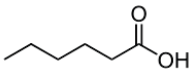
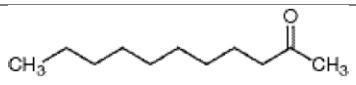
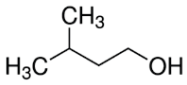
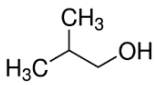
References: [1]Guth, 1997, [2]Campo *et al.*, 2006, [3]Buttery *et al*., 1988 and [4]Ferreira *et al*., 2000.

Aliphatic compounds

Aliphatic volatile compounds may originate from fatty acid oxidation and amino acid degradation (Baumes *et al.*, 2002). Major aliphatic volatile compounds in grapes are the C_6 aldehydes and alcohols derived from fatty acid catabolism (Baumes *et al.*, 2002). Aliphatic volatile compounds are characterized by green or leaf-like sensory descriptors (Table 1.6). During the vinification process, aliphatic compounds are depleted and converted to alcohols and esters and may result in positive sensory attributes (Dunlevy *et al.*, 2009).

Changes in the concentrations of volatile compounds during ripening differ with variety, making it challenging to determine maturity based on varietal volatiles content (González-Barreiro *et al.*, 2015). For example, varietal aroma of *Fernao Pires,* such as monoterpenoids (nerol, linalool, geraniol and α-terpineol) and C_{13}-norisoprenoids (β-ionone and *trans*-β-damascenone) increased for about three weeks after veraison and decreased sharply after (Coelho *et al.*, 2007). In Cabernet Sauvignon, the levels of polyunsaturated fatty acids decreased during ripening resulting in higher concentrations of C_6 aldehydes and alcohols at the initial stages of maturity, and decrease thereafter (Bindon *et al.*, 2013). In interspecific *Vitis* hybrids Frontenac and Marquette, C_6 alcohol concentration peaked around veraison and decrease towards maturation, whereas C_6 aldehydes increased after veraison (Pedneault *et al*., 2013).

Table 1.6 Structures and aroma descriptors of some key aliphatic compounds found in grape berries.

Compound	Structure	Aroma descriptors
Hexanol		Fusel, green, solventy alcoholic, with nuances of tropical fruits, pineapple, apple and cider and rum (1)
cis- 3-hexenol		Greenery, melon peel, pungent freshness grass (2)
trans- 3-hexenol		Bitter, green odor earthy, fatty (1)
Hexanal		Green, fatty, vegetal, herbaceous, fruity and fresh with woody (3)
trans- 2-hexenal		Strong smell of fresh, clean, fruity green leaf with herbal nuances and spicy grass (4)
cis- 3-hexenal		Grass (5)
cis- 3-hexenyl acetate		Sharp fruity-green, sweet, green banana-like (6)
trans-2-*cis*-6 nonadienal		Green-vegetable odor, cucumber, melon, fat (3)
Hex-2,4 *t*,*t*-dienal		Greenery, fruity, aldehyde, citrus, paraffin (7)
Decanal		Sweet, aldehyde, orange, paraffin, citrus zest (4)
Heptanal		Fresh, aldehyde, fat, greenery, penetrating fruity odor (4)
Hexanoic acid		Acidic, burnt, fatty, leather, Sour, cheese (8)
2-Undecanone		Paraffin, fruity, ketonic with pineapple nuances (4)
3-Methyl-1-butanol		Mild, choking alcohol, odor fruity, banana, molasses (8)
2-Methyl-1-propanol		Ethereal, vinous (8)

References: [1]Noguerol-Pato *et al.*, 2014, [2]Ferreira *et al*., 2000, [3]Malherbe *et al.*, 2012, [4]Buttery *et al*., 1988, [5]Buttery *et al.*, 1990, [6]Khiari *etal*., 1995, [7]Teranishi *et al*., 1974 and [8]Rodríguez-Bencomo *et al.*, 2011.

1.3 Abiotic conditions affecting berry biochemistry and ripening

1.3.1 Temperature (GDD)

Grape berries are constantly exposed to several biotic and abiotic factors that affect their development and metabolism (Wu *et al.*, 2019). Among these, temperature is regarded as the most important climatic variable affecting phenology and berry composition (Jarvis, 2017). In order to assign a temperature-driven strategy for the phenological events of plants, GDD has been introduced to assign a heat value to each day (Zhou and Wang, 2018; Luby and Hegeman, 2013).

Growing degree day with mean air temperature is calculated as the average of minimum and maximum daily temperatures as follows (Cushnahan, 2016), where T_i is the mean air temperature (°C) on the i^{th} day of the growing season, where i = 1, 2, … m days with a temperature higher than the base or threshold temperature (T_{base}, base temperature used for grapevine is usually 10°C) during the growing season, and T_{max} and T_{min} are the daily maximum and minimum air temperatures (°C), respectively (Grigorieva *et al.*, 2010).

$$\mathrm{GDD} = \sum_{i=1}^{m}(T_i - T_{\mathrm{base}})$$

$$T_i = (T_{\max} + T_{\min})/2$$

Regional temperature impacts the chemical composition of ripening berries. At higher environmental temperatures, grapes ripen earlier than anticipated, resulting in lower concentrations of amino acids, anthocyanins (Kuhn *et al.*, 2014), thiol-related aroma precursors (Chang *et al*., 2014) and acids compared to those from cooler areas (Deloire, 2014; Serrano *et al.*, 2017). On the other hand, too little heat can delay ripening, leading to wines with low alcohol and poorly developed flavour profiles (Jarvis, 2017). In this respect, Jones and Davis (2000) results showed that an increased number of days during

floraison and veraison, with day maximum temperatures higher than 30°C, were positively correlated to berry quality by influencing early growth events and improving maturation.

According to the findings of Wu (2019), high temperature have a limited impact on primary metabolites, such as sugars and organic acids but significantly reduces certain thiol-related aroma precursors, thus affecting wine quality. High nighttime temperature increases the proportion of assimilated carbon lost through respiration, thus reducing the total amount of sugar available for the clusters (Wu et al., 2019).

The concentration of organic acids at harvest is usually higher, and the pH lower during cool growing seasons than during warm seasons, mainly because lower temperature decrease malate respiration (Ruffner *et al.*, 1984; Alem *et al.*, 2019). Thus, Pereira *et al.* (2006) showed that warm growing seasons might be associated with lower acidity.

The impact of temperature on aroma and flavour compounds is not well understood, yet there is considerable research on this topic in *V.vinifera* varieties. For instance, Lacey *et al*., (1991) found that the accumulation of terpenoids in Sauvignon blanc berries shows an optimum from approximately 10 ºC to 20 ºC. However, the fruit monoterpene concentrations may negatively correlate with the average daily maximum temperature over the ripening period (Marais *et al*., 2017). Within a certain range, the accumulation of C_{13}-norisoprenoids such as *β*-damascenone or *β*-ionone appears to be relatively insensitive to temperature (Keller, 2015). Higher daytime temperatures (but not exceeding 30 °C) allow higher primary metabolism, whereas low nighttime temperatures slow down night respiration and ripening pace, thereby preserving the most delicate grape aromas (Loreto and Schnitzler, 2010). Cooler climate wine tends to have more vegetative compounds such as methoxypyrazines (Sidhu *et al.*, 2015) whereas warm climate wine tend to have less peppery (related to rotundone levels) and more fruity aromas (related to C_{13}-norisoprenoids) (Koundouras, 2018).

1.3.2 Other abiotic parameters

Among the major factors influencing wine character and quality, soil characteristics, water availability, solar radiation and viticultural practices play an important role next to climate and environmental temperature (Teixeira *et al.*, 2014). It is often difficult to separate the influence of one of these factors from the other ones.

For instance, the soil type has been identified as less important for wine aroma when compared to climate or variety. Indeed, soil depth, water-holding capacity and drainage are more important than soil composition (Koundouras *et al.*, 2006). Fruity wine aromas are usually enhanced under moderate soil fertility and water (stony or sandy soils with good drainage) while more vegetative and spicy aromas are more expressed in wines from deep and rich soils with nitrogen and water reserves (Koundouras, 2018). For white cultivars (especially early ripening ones), the best aroma expression is obtained when water and nitrogen are less limiting (Des Gachons *et al.*, 2005).

Vineyard cultivation practices such as training system affect sunlight exposure and grape yield (Reynolds and Vanden Heuvel, 2009). If the extent of pruning is too light, there may be too many shoots leading to dense canopies. On the other hand, if pruning is too severe, the few remaining shoots may grow too vigorously and produce too many laterals, leading to shading in the fruiting zone (Keller, 2010). When considering carbon partitioning, effective pruning is essential to maximize carbohydrate partitioning to fruits and increase the vine's capacity (Fisher, 2009). Sunlight seems to increase the concentration of terpenols (Friedel *et al.*, 2016) and phenols, and extreme exposures tend to reduce the concentration of methoxypyrazines (Pascual *et al.*, 2017; Koundouras, 2018).

1.4 Wine aroma and sensory perception

The profile of volatile compound is one of the most important factors determining the flavour and quality of wine, and largely influences consumer preferences (Chang *et al*., 2014). Although volatile compounds only make up 0.1% v/v of the matrix, the resulting aroma is the most contributing factor to the sensory perception of wine. Some of the aroma compounds are released directly from the grape berries (primary), while others are released during the process of fermentation (secondary) and wine ageing (tertiary) (Jiang and Sun, 2019). Over 1000 volatile compounds have been identified in wine, but only a tiny proportion of these compounds contribute to the perceptible wine aroma (Savits, 2014). This contribution depends on the concentration and the perception threshold of each volatile compound, and the whole wine matrix composition (Chang *et al.* , 2014).

1.4.1 Aromas derived from grapes (varietal aromas)

Grapes contain several systems or pools of precursors categorized as aromatic precursors, including berry free and glycosides of volatile compounds and non-aromatic precursors, including dimethyl sulfide (DMS) precursors, glutathionyl and cysteinyl precursors, fatty acids and Strecker amino acids. These precursors play an essential role in developing wine aroma during fermentation and ageing (Ferreira and Lopez, 2019) (Figure 1.3).

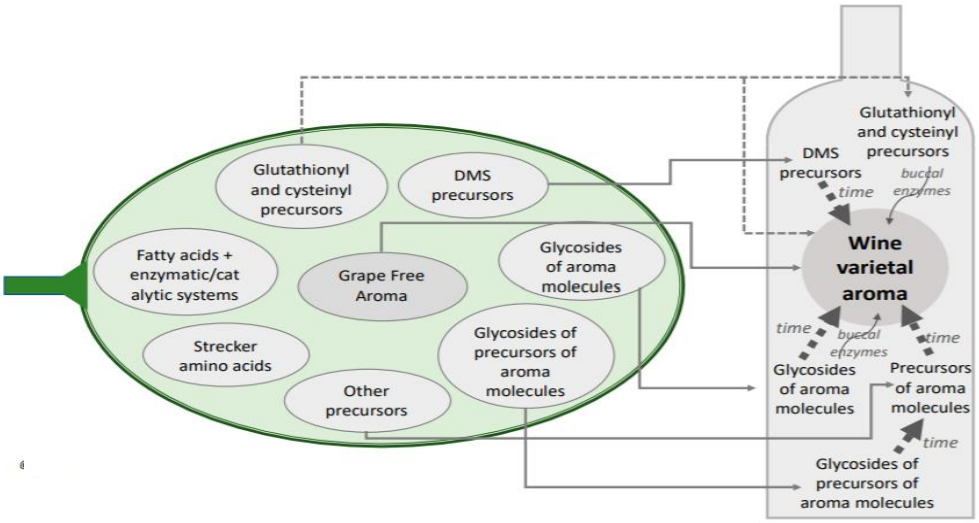


Figure 1.3 The main biological paths in grape involved aroma precursors and their involvement in the development of wine varietal aroma and flavour (Reproduced from Ferreira and Lopez, 2019).

1.4.2 Fermentation aroma

Fermentation derived compounds includes ethyl esters, acetate esters, higher alcohols, volatile acids, and aldehydes (Cheng *et al.*, 2015). Those compounds are synthesized by the yeast during fermentation. Their production is not directly related to the central carbon metabolism but relates to the secondary metabolism of amino acids and fatty acids. Hence, their concentration level is dependent on must composition, including amino acid profile and concentration, fermentation temperature and oxygen exposure (Anke 2013). Fermentation contributes to wine aroma by several mechanisms: firstly by utilizing grape juice components and transforming them into volatile compounds, secondly by producing enzymes that release bound volatile compounds into free volatile compounds, and lastly by the *de novo* synthesis

of primary (ethanol, glycerol, acetic acid, and acetaldehyde) and secondary metabolites (esters, higher alcohols, fatty acids) (Styger *et al*., 2011) (Figure 1.4).

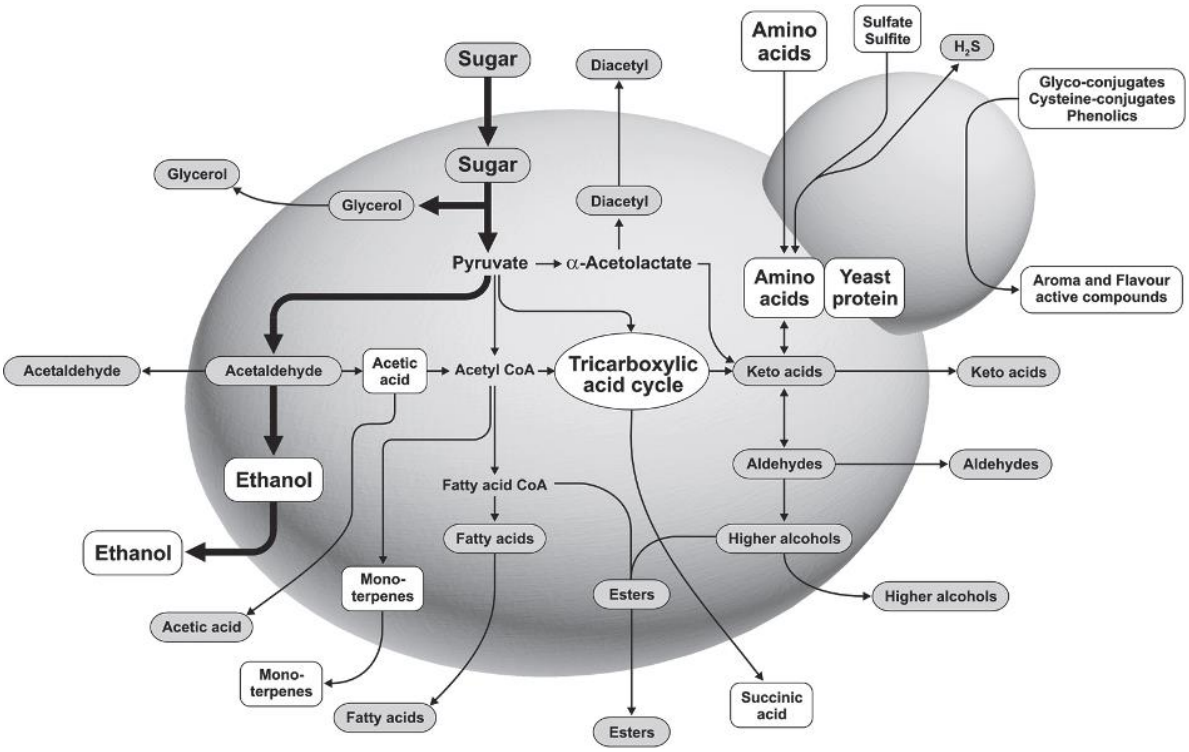


Figure 1.4 Schematic representation of derivation and synthesis of free volatile compounds from sugar, amino acids, and sulfur metabolism by wine yeast, *Saccharomyces cerevisiae* (Reproduced from Vilanova *et al.*, 2007).

1.4.3 Aromas from wine ageing

The composition of wine changes continuously during storage due to the combined influence of storage temperature, oxygen content and storage time (Hernanz *et al.*, 2009). During wine ageing, many reactions occur that cause a significant effect on the organoleptic properties of wine. The most obvious change being wine colour, which refers to a change in phenols profile (Kalkan and Dündar, 2017). The total volatile concentration decreased progressively during storage mainly due to the loss of alcohols. For instance, Hernanz *et al.* (2009) showed that the levels of carbonyl compounds (acetaldehyde, furfural and 5-hydroxymethyl furfural) decreased while the concentration of acids and esters has been increased in Zalema and

Colombard wines in Spain. Oxidation plays an important role in volatile and nonvolatile concentrations in bottle-aged wines as it causes the conversion of ethanol into acetaldehyde, and its conjugates with tannins or anthocyanins (Lambropoulos and Roussis, 2007). This phenomenon results in an oxidative aroma and the disappearance of fruity and flavours produced during fermentation (Liu *et al.*, 2016; Styger *et al*., 2011).

1.5 Analysis of volatile compounds in wine

1.5.1 Gas chromatography and techniques for extraction of volatile compound

The volatile compounds responsible for aroma are present in trace amounts in grapes and wine, making them challenging to extract, identify and quantify (Sánchez-Palomo *et al.*, 2009). As a result, different wine and must aroma extraction and concentration methods have been established using either solid sorbents, cryogenic pre-concentration, membrane devices, solvent extraction, static and dynamic headspace, solid-phase micro-extraction (SPME), stir-bar sorptive extraction (SBSE), supercritical fluid extraction (SFE) and distillation and sublimation techniques (Dewulf *et al*., 2002).

Classical liquid–liquid extraction (LLE) based on organic solvent extraction is one of the most popular methods in the literature for isolating volatile compounds in musts. It can be used for all volatile compounds as low, medium and high volatility in one extraction step. Nevertheless, LLE requires large amounts of high-purity solvents, and it is also a relatively tedious and time-consuming technique (Coleman, 1963).

Steam distillation-solvent extraction technique (SDE) has been applied to grape juice to extract volatile aroma components, with some limitations such as low recovery and loss and/or thermal degradation of specific compounds (Sánchez-Palomo *et al.*, 2009).

SPME is a rapid, simple, and solvent-free sampling technique that allows the pre-concentration of volatile samples. It is growing in popularity due to its ease of use, good sensitivity and low cost (Ouyang and Pawliszyn, 2006). SPME has been successfully applied to determine a variety of compounds in wine (Hórak *et al.*, 2010; Slegers *et al.*, 2015; Slegers *et al.*, 2017) and grape juice (Slegers *et al.*, 2015; Slegers *et al.*, 2017; Pedneault et al., 2013).

1.5.2 Solid-phase extraction (SPE)

SPE can be directly applied to isolate and concentrate volatile compounds from liquid samples. Depending on the type of sorbent and the characteristics of the analyte, a series of physical and chemical interactions are established that allow the analyte of interest to be separated from the rest of the components of the sample. The sorbent utilized to extract volatile compounds is typically a non-polar stationary phase (Castro *et al.*, 2008). The sample's separation, purification, and enrichment are achieved mainly by the selective adsorption and desorption process of the sample components by the solid phase (Mitra, 2003)(Castro *et al.*, 2008).

This technique uses the principle of selective adsorption and selective elution. The more commonly used method is to pass a liquid sample through an adsorbent, retain the substance to be tested, wash away the impurities using a solvent with appropriate strength and elute the tested substance with a small amount of appropriate solvent, thereby achieving three steps of rapid separation, purification and concentration (Cabredo-Pinillos *et al*., 2004).

1.6 Research hypothesis

Our project aimed at investigating the relationships between berry volatile composition with wine volatile composition of three interspecific hybrid *Vitis* sp. harvested in Nova Scotia at three ripening stages. Thus, the following hypotheses have been enunciated:

- The maturity of berries will favorably impact the concentration of free and bound volatiles such as monoterpenes in the interspecific hybrid grape varieties Seyval blanc, L'Acadie blanc and Osceola Muscat by resulting in more favorable aroma profile in final wines.
- Warmer season in Nova Scotia will positively influences the concentration of monoterpenes and C_{13}-norisoprenoids in berries and wines produced from white interspecific hybrids Seyval blanc, L'Acadie blanc and Osceola Muscat.
- Varietal volatile compounds present in hybrid grape varieties such as monoterpenes (linalool geraniol and nerol) and fatty acid degradation (hexanol, *(Z)*-3-hexenol) products will be strongly correlated to the presence of these compounds in wines.

1.7 Research objectives

General objective

To understand the impact of the berry stage of maturity on the volatile compounds of white interspecific hybrid grapes Seyval blanc, L'Acadie blanc and Osceola Muscat and wines produced in Nova Scotia, over two seasons.

Specific objectives

- Understand the impact of different maturity stage and growing-degree days accumulation on the accumulation of free and bound volatile compounds in berries produced from hybrid grape varieties grown in Nova Scotia.
- Understand the impact of different maturity stage and growing-degree days accumulation on the free volatile compounds profile of wines produced from hybrid grape varieties grown in Nova Scotia.
- Relate grape volatile composition to the chemical characteristics and volatile compounds of wines through multivariate statistical analyzes.

2. Impact of harvest date on the chemical composition of berries and wines produced from interspecific *Vitis* sp. cultivars grown in Nova Scotia, Canada over two seasons

This chapter presents our study in the form of a scientific article, of which I am the main author, supported by my co-director, Dr. Pedneault, my director, Dr. Dorais, Dr. Nicolle and Francisco Javier Campos Arguedas. The article is written in English and entitled, "*Impact of harvest date on the chemical composition of berries and wines produced from interspecific Vitis sp. cultivars grown in Nova Scotia, Canada over two seasons*" will be submitted to the journal Oeno One). The implication of each of the authors in this article is as follows: Kumuduni Lakmali: Master's candidate, participation in planning and conducting experiments, analysis of results, and scientific writing of article. Dr. Karine Pedneault: codirector of master's principal investigator, development and ideation of the project, student supervision, correction and revision of the manuscript. Dr Martine Dorais: supervisor, collaboration with the project, correction and revision of the manuscript. Dr. Paméla Nicolle: development of winemaking protocol, berry aroma analytical method and manuscript revision. Francisco Javier Campos Arguedas: integration of chromatograms for determination of grape volatile compounds.

2.1 Résumé

Les provinces maritimes, dont la Nouvelle-Écosse, présentent des conditions climatiques très variables qui peuvent conduire à différents niveaux de maturité et de qualité des baies. La relation entre la maturité des baies et la composition chimique du vin a été étudiée chez les cultivars de *Vitis vinifera*, mais peu d'études chez l'hybride interspécifique *Vitis sp*. ont été conduites. Dans ce contexte, notre projet visait à étudier la relation entre la composition des composés volatiles des baies et la composition des composés volatiles présents dans le vin de trois hybrides interspécifiques *Vitis sp*. récoltés en Nouvelle-Écosse à trois stades de maturation. Nos résultats ont montré que certaines catégories de composés volatils tels que les produits de dégradation des acides gras diminuaient avec la maturation, tandis que certains autres composés comme les terpènes et les norisoprénoïdes en C_{13} augmentaient. Les connaissances acquises sur la composition volatile de ces cépages et sa relation avec les vins permettront de sélectionner une maturité de récolte adaptée pour les cépages hybrides afin d'optimiser la qualité aromatique des vins produits.

2.2 Abstract (English)

Canadian Maritime Provinces such as Nova Scotia show highly variable climatic conditions that can lead to different levels of berry ripeness and quality. The relationship between berry ripeness and wine chemical composition has been studied in *Vitis vinifera* cultivars but little investigation in interspecific hybrid *Vitis sp*. In this context, our project aimed at investigating the relationships between berry and wine volatile composition of three interspecific hybrid *Vitis* sp. harvested in Nova Scotia at three ripening stages in 2019 and 2020. Our results showed that specific categories of volatile compounds, such as free fatty acid degradation products, decreased in berries with the ripening, while some other compounds like bound terpenes and C_{13}-norisoprenoids increased. Grape ripening stage significantly impacted the accumulation of wine volatile as aromatic esters (phenethyl acetate), monoterpenes (β-linalool), fatty acid ethyl esters, furaneol and γ-butyrolactone. Knowledge acquired on the volatile composition of these grape varieties and their relation to wines make it possible to select suitable harvesting maturity for hybrid grape varieties to optimize the aromatic quality of the wines produced.

2.3 Introduction

Wine production in cold climate has grown significantly with the arrival of interspecific hybrid grape varieties, that are well suited for short growing seasons and cold winters (Lewis, 2008b). The Atlantic province of Nova Scotia, Canada, shows variable yearly climatic conditions, making grape production challenging with sudden changes in weather conditions, heavy rains, frost, and high winds, especially during grape veraison (Lewis, 2008a).

Understanding the impact of harvesting date on the chemical composition of berries and then wine is of tremendous importance to uplifting the viticulture and optimizing wine quality. Yet, predicting the optimal harvest date is still difficult, since it is strongly dependent on the year's climatic conditions and environmental factors such as solar radiation, temperature, and water availability (Martinon *et al.*, 2015). In cold climate regions, limited accumulations of heat units during the growing season often lead to insufficient ripening of grape varieties, resulting in high fruit acidity levels at harvest (Pedneault *et al*., 2013). Moreover, under northern climate, it is often difficult to identify the best harvesting maturity with the aid of traditional maturity assessment parameters such as total soluble solids (TSS) and acidity (Gustafsson and Mårtensson 2005). Although the sugar content may not be proper for harvesting, berries can have a high concentration of interesting varietal aromas and flavours (Bremmer, 2010). Therefore, analysis of varietal aroma compounds (terpenes, C_{13}-norisoprenoids and C_6-compounds) can be used as a proper criteria for selecting the harvest date (Chang *et al*., 2014). It has been reported that aromatic maturity was best assessed using the ratio of *Z*-3-hexenol to *trans*-2-hexenal, which showed a constant decrease until maturity in Frontenac and Marquette grape berries (Pedneault *et al*., 2013), Shine Muscat (Wu *et al.*, 2020) and Cabernet Sauvignon grape berries (Gao *et al.*, 2019). It has been shown that the concentration of some volatile compounds such as methyl hexanoate, 1-nonanal, benzaldehyde, rose oxide, and linalool often increases during berry development (Maoz *et al.*, 2017, Fang and Qian, 2006) and conversely, the concentration of other volatile compounds, such as geraniol, (*Z*)-3-hexen-ol, (*E*)-2-octenal and 1-pentanal decrease during berry development (Kalua and Boss, 2010).

The relationship between berry ripeness and wine chemical composition has been studied in *Vitis vinifera* cultivars (Gao *et al.*, 2019; Bindon *et al.*, 2013; Zhao *et al.*, 2019), but few investigations were performed on interspecific hybrid *Vitis sp*. varieties. In recent studies, Slegers and coauthors found a clear relationship between the occurrence of free C_6 compounds and monoterpenes in grape berries and their presence in the resulting wine, both in red and white hybrid varieties harvested at full ripeness (Slegers *et al.*, 2015, Slegers *et al.*, 2017). On the other side, studies exploring the impact of berry maturity on wine chemical composition are very scarce in interspecific hybrid varieties.

In this context, this project aimed at investigating the relationship between the chemical composition of berries and wines made from interspecific hybrid white grapes L'Acadie blanc, Osceola Muscat and Seyval blanc harvested at three maturity stages and different growing degree days (GDD) in Nova Scotia, Canada over two vintages 2019 and 2020. We hypothesized that the maturity of berries and the seasonal climatic conditions impact the concentration of free and bound volatiles in the hybrid grape varieties, which strongly correlate with the varietal aroma compounds measured in the wine. To test these assumptions, we have characterized the free and bound volatile compounds profiles of berries and free volatile compounds in the respective wines and performed redundancy analysis (RDA) for relating berry grape volatiles with their wine volatile compounds.

2.4 Methodology

2.4.1 Experimental design and berry sampling

Interspecific white *Vitis* sp. Osceola Muscat, Seyval blanc and L'Acadie blanc were harvested from a commercial vineyard located in the Gaspereau Valley, Nova Scotia (45°04'20.2"N 64°17'43.5"W) from September to October in consecutive vintages 2019 and 2020. Vineyard vines were spur pruned to 12 buds per vine and trained in a vertical shoot positioning system. Row spacing and vine spacings were 2 to 3 m and 1.20 m, respectively. Cluster thinning was conducted at developmental stage EL-32 (beginning of bunch closure and berries touching; Lorenz *et al.*, 1995). For each variety, berry samples were harvested at three maturity stages (EL-37, EL-38 and EL-39) corresponding to 918, 928 and 935 GDD in 2019 and 1034, 1083 and 1132 GDD in 2020.

Each sample (30 to 50 kg) was harvested on 2 to 6 subplots (e.g., 4 to 5 plants per subplot), as shown in Table 2.1. Berries for chemical analysis (5 to 6 clusters) were transported from the field to the lab well packed with ice packs inside cool retaining containers. These 5 to 6 clusters were used to measure physiological properties (berry and cluster weight); fifty randomly picked berries were used for berry physicochemical analysis (TSS, TA and pH) and the remaining was stored at −18 °C for further analysis of free and bound volatile compounds. Berries for winemaking (30 to 40 kg per replicate) were sulfated with 2 g potassium metabisulphite per 100 kg of grapes at the vineyard and transported from the field to the research winery at ambient temperature (±15 °C) in plastic crates and stored at 4°C until winemaking (on the same day in 2019 and the next day in 2020).

Table 2.1 Harvest plan for 2019 and 2020 vintages. The rows 1, 2 and 3 in table represent consecutive three rows in the vineyard and each row consisted of 25 subplots (separated sections along a row in the vineyard) numbered 1 to 25. Each subplot consisted in 4 or 5 grape plants. Berries harvested at three maturity stages (EL-37, EL-38 and EL-39) corresponding to 918, 928, and 935 GDD (3rd, 10th and 22nd of October) in 2019 and to 1034, 1083 and 1132 GDD in 2020 (15th, 28th of September and 8th of October).

Cultivar	Year	Harvesting date	Replicate	Row 1 (Subplot No)	Row 2 (Subplot No)	Row 3 (Subplot No)
OM (Osceola Muscat)	2019	1	1	4, 16	-	-
			2	1, 11	-	-
			3	7, 10	-	-
		2	1	2, 17	-	-
			2	5, 14	-	-
			3	3, 15	-	-
		3	1	8, 12	-	-
			2	6, 18	-	-
			3	9, 13	-	-
AC (L'Acadie blanc)	2019	1	1	3, 17	13, 21	-
			2	1, 12, 19	16	-
			3	4, 13, 22	22	-
		2	1	2, 11, 21	23	-
			2	9, 18	14, 17	-
			3	5, 10, 23	18	-
		3	1	7, 16, 20	20	-
			2	6, 14	15, 24	-
			3	8, 15, 24	19	-
SB (Seyval blanc)	2019	1	1	4, 12	3, 17	-
			2	1,10	2,13	-
			3	6, 13	7, 18	-
		2	1	5, 17	6, 12	-
			2	2, 15	8, 10	-
			3	7, 14	1, 15	-
		3	1	3, 11	4, 14	-

			2	9,16	9, 11	-
			3	8, 18	5, 16	-
OM (Osceola Muscat)	2020	1	1	3, 13	9	-
			2	8, 20	16	-
			3	5, 16	3	-
		2	1	9, 17	12	-
			2	7, 15	7	-
			3	10	13, 21	-
		3	1	11, 21	11	-
			2	2, 19	5	-
			3	24	2, 22	-
AC (L'Acadie blanc)	2020 15/September	1	1	6, 18	4,13	-
			2	10, 15	8, 16	-
			3	11, 17	6, 15	-
		2	1	8, 16	9, 17	-
			2	5, 14	10, 19	-
			3	4, 13	5, 18	-
		3	1	9,7	2, 14	-
			2	2, 12	7, 12	-
			3	19, 3	3, 11	-
SB (Seyval blanc)	2020	1	1	4, 13	8, 14	5, 15
			2	6, 16	11, 17	3, 7
			3	3, 8, 14	7, 12, 16	
		2	1	5, 10	5, 13	13, 16
			2	7, 12	3, 15	10, 4
			3	11, 18	6, 10, 18	12
		3	1	1, 15, 19	4	9, 19
			2	9	1	2, 6, 8, 18
			3	2, 17	9, 2	11, 14

The numbers indicated for each row are the subplot that has been sampled. And subplot has been randomized allocated to the harvesting dates.

Daily minimum and maximum field temperature were taken from the monthly climate report of Environment and Natural Resources of the Government of Canada (Monthly Climate Summaries, (2020) Kenville, NS (45.0769° N, 64.4945° W) (https://climate.weather.gc.ca).

Growing degree day with mean air temperature was calculated using the average of minimum and maximum daily temperatures as follows (Cushnahan, 2016):

$$\mathrm{GDD} = \sum_{i=1}^{m} (T_i - T_{\mathrm{base}})$$

$$T_i = (T_{\max} + T_{\min})/2$$

where T_i is the mean air temperature (°C) on the i^{th} day of the growing season (i = 1, 2, … m) with a temperature higher than the base or threshold temperature (T_{base}, 10 °C) during the growing season, and T_{max} and T_{min} are the daily maximum and minimum air temperatures (°C), respectively (Grigorieva *et al.*, 2010).

2.4.2 Reagents and Standards used for the different analyses and winemaking

Absolute ethanol (97%), methanol (HPLC grade), dichloromethane (HPLC grade), and n-Hexane (99%) were purchased from Sigma-Aldrich (St. Louis, MO). *n*-Pentane (HPLC grade) and citric acid (anhydrous) were purchased from Anachemia (Mississauga, ON, CA). Sodium sulfate anhydrous was purchased from VWR (Solon, OH, CA), and sodium phosphate dibasic were purchased from Spectrum Chemicals (Gardena, CA, USA). Insoluble poly vinyl poly pyrrolidone (PVPP) was purchased from Millipore Corporation (Billerica, MA, USA). Internal standards (+/-)-2-octanol (>99.5%) and Nonyl ß-D-glucopyranoside (>97%) were purchased from Sigma Aldrich (Oakville, ON, CA).

2.4.3 Basic physicochemical analysis of grape juice

Fifty randomly selected berries from 6 to 10 clusters were manually crushed. The juice was recovered by sieving and analyzed for total soluble solids, pH, and total titratable acidity with the use of a hand refractometer (Atago, Fukuoka, Japan), a pH meter (MP 220, Hanna Instruments, Darmstadt, Germany) and a titratable acidity meter (HI 84502, Hanna Instruments, Woonsocket Rhode Island, USA). Titratable acidity was recorded in g/L tartaric acid equivalent. Ammonia and primary amino nitrogen content were measured by UV-Visible spectrophotometry using the enzymatic test kit UniTab Reagent (Unitech scientific California, USA).

2.4.4 Winemaking

Berries were destemmed and crushed, and the juice was recovered by pressing at 1.8 bars. Juices were sulphated by adding 1 mL of a 5% SO_2 solution per liter of grape juice and juice was transferred into 30 L plastic fermenters for a total of 27 fermenters per year (3 harvesting dates x 3 varieties x 3 replicates). A concentration of 40 µL/L of juice of Lysis elite enzyme (Sas sofralab, Magenta, France) was added 1-hour after SO_2 addition and juice clarify for 12 hours at 15 °C. After clarification, juices were racked and nitrogen content was measured using AMM-150 Reagent Kit, and PAN-150 reagent kits. When required, nitrogen content was adjusted by adding Vivactiv Premier (Sas Sofralab, Magenta, France) (0.4 g/L) and Vivactiv Performance (Sas Sofralab, Magenta, France) (0.4 g/L). Alcoholic fermentation was induced by activated dry yeast *Saccharomyces cerevisiae*e (Selectys L'eclatante, Sas

Sofralab, Magenta, France) (0.2 g/L) and carried out at 18 °C until the dryness. The progression of alcoholic fermentation was monitored daily by measuring the specific gravity and the temperature. In 2019, the temperature was regulated by setting a room temperature, while in 2020, Immersion Pros (Brewjacket, Boulder, Colorado) were installed on each fermenter to maintain juice temperature. At the end of alcoholic fermentation wine was racked into 23 L glass carboys equipped with airlocks, clarified by adding Bentogreen (Sas Sofralab, Magenta, France) (0.5 g/L of wine), and SO_2 of wine was adjusted to 40 mg/L. After two weeks, clarified wine was racked and cold stabilized with the addition of a cream of tartar (4 g/L of wine) at 0 °C. The wines were racked after 2 weeks at 18 °C, and their level of free SO_2 was readjusted to 40 mg/L. Wine was then filtered (polished and sterilized), bottled and stored at 4 °C for 6 months until further analysis.

2.4.5 Free and bound volatile analysis of grapes

Grape free and bound volatile compounds were extracted according to the procedures described by Crespo *et al*. (2018) and Lanaridis *et al*. (2002), with some modifications. In detail, around 500-600 g of frozen destemmed grapes were weighted and thawed overnight at 4 °C. Berries were then ground and the mixture was filtered. The filtrate was centrifuged at 10,000 rpm, at 4 °C, for 10 minutes and the precipitate was removed by filtering through a ball of cotton wool. Polyvinyl poly pyrrolidone was added (PVPP of 1 g/100 g of juice) to the filtrate, stirred for 20 minutes and followed by successive vacuum filtration on Whatman grade 4, 3, and 5 filter papers (particle retention of 25 µm, 6 µm and 2.5 µm). A fraction of 100 mL of filtered grape juice was diluted with 100 mL of deionized water and two internal standards of 100 µL of 2-octanol (230 mg/L in ethanol) and 150 µL of nonyl *ß*-D glucopyranoside (1000 mg/L in 50:50 ethanol/water) were added. Volatiles compounds were extracted by solid-phase extraction (SPE) using 500 mg (6 mL) Isolute ENV + polymer cartridges (Biotage, Charlotte, NS, USA). Cartridges were conditioned by passing 20 mL of methanol and 20 mL of distilled H_2O at a flow rate of 1 mL/min. Samples were loaded at the same flow rate. After passing the samples, cartridges were rinsed with 50 mL of deionized water. Free and bound volatile compounds were eluted with 25 mL of dichloromethane and 25 mL of methanol, respectively at a flow rate of 1mL/min.

Bound volatile compounds were concentrated under a nitrogen flow at 45 °C to dryness. Residue was solubilized by 500 µL of phosphate: citrate buffer (1:1; v/v 0.2 M sodium hydrogen phosphate/0.1 M citric acid, pH 5.0) and vortexed. Two hundred µL of an enzyme solution (AR2000) (70 mg/mL in a 1:1 v/v 0.2 M phosphate/ 0.1 M citrate buffer solution; pH 5.0) was added to the mixture and kept at 40 °C for 24 h. After hydrolysis, 25 µL of 2-octanol (230 mg/L in ethanol) was added and the released volatile were recovered 3 times by extraction using 2 mL of pentane/dichloromethane (2:1, v/v). The organic phase was dried with anhydrous sodium sulfate and concentrated using a Vigreux column (Kuderna-Danish) down to 200 µL at 35 °C. The organic extract was then collected into glass vials and stored at −20 °C until GC-MS-FID analysis.

The free fraction was dried with anhydrous sodium sulfate and concentrated down to 100 µL at 35 °C using a Vigreux column (Kuderna-Danish) and the volume was adjusted to 500 µL using hexane. The organic extract was then collected into glass vials and stored at −20 °C until used for GC-MS-FID analysis.

Free and bound volatile fractions were injected on an Agilent 7890A gas chromatograph (Santa Clara CA, USA) coupled to an Agilent 5975 mass spectrometer and a flame ionization detector (GC-MS-FID), using an HP-5MS Ultra inert column (30 m×250 µm× 0.25 µm I.D.) (Agilent Technologies, Santa Clara, CA, USA). Each sample (1 µL) was injected into the GC in splitless mode. The injector temperature was maintained at 250°C. The carrier gas was helium. The temperature program was as follows: initial temperature, 40 °C for 4 min and then ramped at a rate of 3.5 °C/min to 240 °C, and 20 °C/min to 250 °C. Peaks were identified using authentic standards, retention indexes and the NIST Mass spectral library (Gaitherburg, MD, USA), and semi-quantified against the internal standard 2-Octanol as described by (López *et al.*, 2002a).

2.4.6 Wine physicochemical analysis

Three samples of wines were per replicate were analyzed for pH, and total titratable acidity with the use of a pH meter (MP 220, Hanna Instruments, Darmstadt, Germany) and a titratable acidity meter (HI 84502, Hanna Instruments, Woonsocket Rhode Island, USA). The total and free sulfur dioxide content was measured using SO_2 mini titrator (HI84500, Hanna

Instruments, Woonsocket Rhode Island, USA). The alcohol percentage of wines was estimated using a wine hydrometer (Mosti Mondiale, QC) (Gao *et al.*, 2019).

2.4.7 Wine free volatile analysis

With some modifications, free volatile compounds of wine were extracted and determined as described by López et al. (2002). Wine bottles were opened on the day of analysis, 100 mL wine samples were treated with polyvinyl poly pyrrolidone (PVPP of 5 g/L of wine). The solution was stirred for 20 minutes, centrifuged at 5000 rpm for 10 minutes and then filtered on 25 µm filter papers (Whatman No 4, Darmstadt, Germany). A 25 mL fraction of the filtrate was diluted with 25 mL of deionized water. 30 µL of 2-octanol (500 mg/L of ethanol) was added as the internal standard. Wine volatile was extracted by solid-phase extraction (SPE) using 500 mg C_{18} Resin Cartridges (Biotage, Charlotte, NS, USA). Cartridges were conditioned with 15 mL of methanol and then 15 mL of deionized water at a 1 mL/min flow rate. Samples were loaded, and the cartridges were rinsed with 25 mL of distilled water at a 1 mL/min flow rate. Free volatiles were eluted with 15 mL of pentane: dichloromethane (2:1). The extracted organic phase was dried with anhydrous sodium sulfate, concentrated to 200 µL using Vigreux column (Kuderna-Danish) at 35 °C. The organic extract was then collected into glass vials and stored at −20 °C until GC-MS-FID analysis.

Wine free volatile were injected on an Agilent 7890A gas chromatography (Agilent Technologies, Santa Clara CA, USA) coupled to an Agilent 5975 mass spectrometer and a flame ionization detector (GC-MS-FID), using an HP-5MS Ultra inert column (30 m×250 µm× I.D. 0.25 µm; Agilent Technologies, Santa Clara CA, USA). Sample (1 µL) was injected into the GC in splitless mode. The injector was maintained at a temperature of 250°C. The carrier gas was helium. The temperature program was as follows: initial temperature, 40 °C for 4 min and then ramped at a rate of 2 °C/min to 120 °C, at a rate of 10 °C/min to 240 °C and, finally, at a rate of 25 °C/min to 250 °C. Peaks were identified using authentic standards, retention indexes and the NIST Mass spectral library (Gaitherburg, MD, USA), and semi-quantified against the internal standard 2-Octanol.

2.4.8 Statistical analyses

Analysis of variance (ANOVA) with a mixed model was performed using R Software (R version 3.6.1; Boca, Raton, FL, USA). Means were compared using Tukey's test at $\alpha = 0.05$.

A redundancy analysis (RDA) was carried out to relate wine volatile compounds (dependent variables) to berry volatile compounds (independent variables), using the R software with the Vegan, ggrepel and ggplot2 packages. Principal Component Analysis (PCA) was performed on the means scores of volatiles of grapes (based on the grape varieties, ripening stages and harvesting vintages) using the R software (R version 3.6.1) (Boca, Raton, FL, USA).

2.5 Results

2.5.1 Crop load, GDD accumulation and variation of average daily temperature

Crop load and GDD accumulated from April to October per harvest dates are shown in Table 2.2 and Table 2.3, respectively. Crop load significantly decreased from harvest 1 to harvest 3 in L'Acadie blanc and Osceola Muscat berries harvested in 2020.

Table 2.2 Crop load per plant (kg/plant) at each harvest (1, 2 and 3) (El-37, EL-38 and EL-39) for three interspecific hybrid grape varieties Seyval blanc, L'Acadie blanc and Osceola Muscat.

Year	**Grape variety**	**Harvesting date and crop load (kg/plant)**		
		Harvest 1	**Harvest 2**	**Harvest 3**
2019	Seyval blanc	1.49±0.38[a] a	1.24±0.31 ab	0.72±0.24 b
	L'Acadie blanc	2.10±0.03 a	1.66±0.15 b	1.90±0.29 ab
	Osceola Muscat	3.48±0.15 a	3.64±0.15 a	3.23±0.71 a
2020	Seyval blanc	1.98±0.60 a	2.24±014 a	1.87±0.41 a
	L'Acadie blanc	3.63±0.21 a	3.50±0.14 a	2.72±0.24 b
	Osceola Muscat	3.81±0.12 a	3.61±027 ab	2.97±0.60 b

[a] Each value represents the mean ± standard deviation of harvest from 6 to 18 subplots with three field replicates. For a given variety and year, values followed by a different letter are significantly different at $P \leq 0.05$ according to Turkey's honestly significant difference test.

Table 2.3 Accumulated GDD at each harvest (1, 2 and 3) for three interspecific hybrid grape varieties in two vintages 2019 and 2020. All varieties were harvested at the same dates.

Year	Harvesting date and accumulated GDD (Based on 10° C)		
	Harvest 1	Harvest 2	Harvest 3
2019	918	928	935
2020	1034	1083	1132

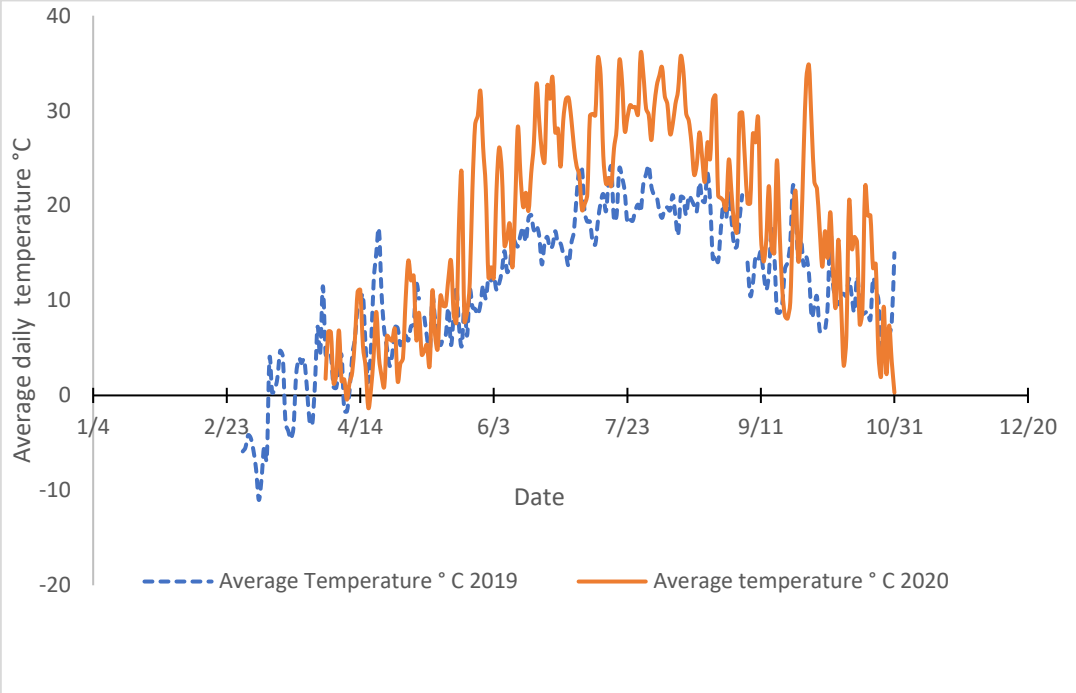


Figure 2.1 Variations of average daily temperature from March to October at Kenville, NS during the two harvesting seasons 2019 and 2020

(Data from: Environment and Natural Resources of the Government of Canada Monthly Climate Summaries, (2020) Kenville, NS (45.0769° N, 64.4945° W) (https://climate.weather.gc.ca).

2.5.2 Berry and wine basic metrics

Berries were harvested at three harvesting stages (H1, H2 and H3) corresponding to 918, 928 and 935 growing-degree days (GDD) in 2019 and 1034, 1083 and 1132 GDD in 2020. The harvest date did not impact berry or cluster weight of L'Acadie blanc, while cluster weight

significantly decreased in both years for Osceola Muscat from 177 to 98.6 g in 2019 and from 84.4 to 55.2 g in 2020 (Table 2.4). In contrast, berry weight and cluster weight of Seyval blanc harvested in 2019 increased significantly from HD1 to HD 2.

For all varieties, total soluble solids content (TSS) significantly increased with ripening, except for Seyval blanc in 2019 (Table 2.4). The TSS of L'Acadie blanc berries significantly increased from 17.5 to 19.6 in 2019 and 17.4 to 21.9 in 2020. Similarly, in Osceola Muscat, TSS increased significantly from 16.5 to 18.8 in 2019 and 18.8 to 22.6 in 2020. On the other hand, the titratable acidity (TA) decreased significantly with ripening in both years and for all varieties, except for Seyval blanc in 2019, where no significant difference was observed. In 2020, TA decreased with ripening by 41%, 43% and 50% in L'Acadie blanc, Osceola Muscat and Seyval blanc. In 2019, TA decreased by 22% and 23% in L'Acadie blanc and Osceola Muscat respectively, whereas Seyval blanc did not show a significant decrease. pH significantly increased with ripening in all three varieties in 2020.

The PAN of L'Acadie blanc, Osceola Muscat and Seyval blanc increased from 72 to 138, 35.3 to 84 and 71.3 to 115 mg/L in 2019 and 78 to 101, 29.3 to 47.3, 55.7 to 113 in 2020, respectively. In 2019, YAN of L'Acadie blanc, Osceola Muscat and Seyval blanc increased significantly with ripening in 2019 from 77.8 to 141, 38.4 to 86.3, 75.2 to 119 mg/L. And in 2020 YAN of L'Acadie blanc, Osceola Muscat significantly increased from 81 to 102 and 30.3 to 48.7 mg/L, while Seyval blanc didn't show a significant increase.

Table 2.4 Fresh berry weight (g), cluster weight (g) total soluble solid content (TSS); °Brix), pH, titratable acidity (g/L, tartaric ac.eq.), primary amino nitrogen content (PAN); (mg/L) and yeast assimilable nitrogen content (YAN); (mg/L) of the interspecific hybrid grapes L'Acadie blanc, Osceola Muscat and Seyval blanc harvested in the Province of Nova Scotia (Canada) at three dates in 2019 and 2020.

	L'Acadie blanc						**Osceola Muscat**						**Seyval blanc**					
Year	2019			2020			2019			2020			2019			2020		
Harvest date	3rd Oct[a]	10th Oct	22nd Oct	15th Sep	28th Sep	8th Oct	3rd Oct	10th Oct	22nd Oct	15th Sep	28th Sep	8th Oct	3rd Oct	10th Oct	22nd Oct	15th Sep	28th Sep	8th Oct
GDD	918	928	935	1034	1083	1132	918	928	935	1034	1083	1132	918	928	935	1034	1083	1132
Berry weight (g)	1.06[b]	1.11	1.23	1.09	1.15	1.14	1.58	1.49	1.72	1.68	1.61	1.69	1.45 ab	1.80 a	1.53 b	1.55	1.67	1.78
Cluster weight (g)	106	122	121	99.9	110	97.3	177 a	105 b	98.6 b	84.4 a	77.0 a	55.2 b	146 b	285 a	177 b	249	364	354
TSS (° Brix)	17.5 b	18.4 b	19.6 a	17.4 b	21.3 a	21.9 a	16.5 b	17.3 ab	18.8 a	18.8 b	20.5 ab	22.6 a	16.7	18.5	18.6	16.1 b	20.6 a	21.8 a
pH	3.10	3.00	2.90	2.80 b	3.20 a	3.30 a	3.00 a	2.90 b	2.90 b	2.70 b	3.10 a	3.20 a	3.10	2.90	2.90	2.80 b	3.10 a	3.70 a
TA (g/L, tartaric ac eq.)	13.0 a	10.0 b	10.0 b	10.9 a	8.60 b	6.40 c	14.3 a	10.7 b	10.9 b	11.2 a	8.40 b	6.30 b	14.4	11.5	10.3	14.4 a	8.5 b	7.2 b
PAN (mg/L)	72.0 b	94.0 b	138 a	78.0 b	98.3 a	101 a	35.3 b	47.9 b	84.0 a	29.3 b	45.7 a	47.3 a	71.3 b	97.7 a	115 a	55.7 b	91.0 ab	113 a
YAN (mg/L)	78.0 b	98.0 b	141 a	81.0 b	100 a	102 a	38.4 b	51.9 b	86.3 a	30.3 b	48.0 a	48.7 a	75.2 b	102 a	119 a	79.7	91.7	114

[a] The first, second and last harvest dates represents EL-37, EL-38 and EL-39 stages corresponding to 918, 928, and 935 GDD in 2019 and to 1034, 1083 and 1132 GDD in 2020.

[b] Each value represents the means of three field replicates (n=3). For a given variety and year, values followed by a different letter are significantly different at $P \leq 0.05$ according to Turkey's honestly significant difference test (Please refer to Supplemental material for interactions statistics and p values).

Table 2.5 Alcohol content (% v/v), titratable acidity (g/L, tartaric ac. eq.) and pH of wines produced from three interspecific hybrid grapes L'Acadie blanc, Osceola Muscat and Seyval blanc harvested in the Province of Nova Scotia (Canada) at three dates in 2019 and 2020.

	Variety																	
	L'Acadie blanc						Osceola Muscat						Seyval blanc					
Year	2019			2020			2019			2020			2019			2020		
Harvest date	3rd Oct [a]	10th Oct	22nd Oct	15th Sep	28th Sep	8th Oct	3rd Oct	10th Oct	22nd Oct	15th Sep	28th Sep	8th Oct	3rd Oct	10th Oct	22nd Oct	15th Sep	28th Sep	8th Oct
GDD	918	928	935	1034	1083	1132	918	928	935	1034	1083	1132	918	928	935	1034	1083	1132
Alcohol % v/v	10.0[b]	10.4	10.8	11.2	10.3	12.0	9.50	10.0	9.90	11.6	12.4	12.5	10.0	10.4	9.80	12.1	12.5	11.5
TA (g/L, tartaric ac. eq.)	10.8 a	9.80 b	9.00 c	10.9 a	8.10 b	7.40 c	11.2a	10.5 a	9.30 b	12.0 a	10.1 b	9.30 c	11.7a	11.2 b	9.90c	13.4 a	10.6 b	9.40 b
pH	2.80 b	2.80 b	2.90 a	2.60 c	2.70b	2.80a	2.70 b	2.70 b	2.80 a	2.50 b	2.60 a	2.70 a	2.60 b	2.70 ab	2.70 a	2.50 b	2.60 a	2.70 a

[a] The first, second and last harvest dates represents EL-37, EL-38 and EL-39 stages corresponding to 918, 928, and 935 GDD in 2019 and to 1034, 1083 and 1132 GDD in 2020.

[b] Each value represents the means of three field replicates (n=3). For a given variety and year, values followed by a different letter are significantly different at $P \leq 0.05$ according to Turkey's honestly significant difference test (Please refer to Supplemental material for interactions statistics and p values).

Wine TA significantly decreased as berry ripened from 10.8 to 9, 11.2 to 9.3 and 11.7 to 9.9 in 2019 and from 10.9 to 7.4, 12 to 9.3 and 13.4 to 9.4 in 2020 for the three varieties L'Acadie blanc, Osceola Muscat and Seyval blanc, respectively (Table 2.5). At the same time, wine pH increased significantly for all three berry varieties from 2.8 to 2.9, 2.7 to 2.8, 2.6 to 2.7 in 2019 and 2.6 to 2.8, 2.5 to 2.7, 2.5 to 2.7 in 2020 for three varieties L'Acadie blanc, Osceola Muscat and Seyval blanc respectively. On the other side, no significant change was observed for the alcohol content of wines.

2.5.3 Free volatile compounds from grapes

Six classes of free volatile compounds were characterized in the berry juice of the studied interspecific hybrid varieties: (1) fatty acid degradation products (FADP), (2) alcohols (AL), (3) fatty acids (FA), (4) fatty acid esters (FAE), (5) volatile phenols and benzene derivatives (VP) and (6) monoterpenes (MT) (Table 2.6).

The FADP accounted for the highest proportion (approximately 93%) of free volatiles in all studied varieties, with (*E*)-2-hexenal as the main FADP, followed by hexanal and (*E*)-2-hexenol. The concentration of FADP in L'Acadie blanc decreased significantly with ripening in 2019, whereas it increased significantly in Osceola Muscat and Seyval blanc with ripening. In 2020, no difference between the first (1034 GDD) and the last harvest date (1132 GDD) was observed. Despite the ripening stage, the concentration of total FADP showed a smaller value in 2020 than in 2019 in L'Acadie blanc berries ($p<0.001$; Table 2.9, Supplemental material). In 2020, high proportions of benzene derivatives, specifically 2-phenylethanol, were detected in Osceola Muscat and Seyval blanc berries. This same year, the concentration decreased significantly in Seyval blanc berries. In 2020, the total concentration of alcohols increased significantly with ripening in Osceola Muscat berries from 16.8 to 71.2 ug/L, while it decreased in Seyval blanc from 83.5 to 16.5 ug/L. Overall, the sum of alcohol in Osceola Muscat and Seyval blanc was higher in 2020 than in2019 (Table 2.9 at supplemental material). FAE were accumulated in Seyval blanc berries with a maximum value of 26.1 µg/L at the first harvest (1034 GDD) in 2020. Free monoterpenes (MT) were only detected in Osceola Muscat and Seyval blanc berries. Osceola Muscat berries showed the highest level (324 µg/L) in 2020 at second harvest (1083 GDD). In addition, regardless of the harvest stage, a higher accumulation of MTs was observed in 2020 in comparison to 2019 in Osceola Muscat berries (Table 2.9 at supplemental material). In Seyval blanc berries, the sum of total monoterpenes increased significantly with ripening from 3.7 µg/L to 25.8 µg/L in 2019.

Table 2.6 Free volatile compounds from the juice of the interspecific hybrid grapes L'Acadie blanc, Osceola Muscat and Seyval blanc harvested in the Province of Nova Scotia (Canada) at three dates in 2019 and 2020

	L'Acadie blanc					**Osceola Muscat**						**Seyval blanc**					
Year	2019			2020		2019			2020			2019			2020		
Harvest date	3rd Oct[a]	10th Oct	22nd Oct	15th Sep	28th Sep	3rd Oct	10th Oct	22nd Oct	15th Sep	28th Sep	8th Oct	3rd Oct	10th Oct	22nd Oct	15th Sep	28th Sep	8th Oct
GDD	918	928	935	1034	1083	918	928	935	1034	1083	1132	918	928	935	1034	1083	1132
Fatty acid degradation products (µg/L)																	
(*E*)-2-Hexenal	4300[b] a	2430 b	2680 b	1230	1190	730 b	640 b	1130 a	990 a	220 b	760 a	1150 b	2410 a	1980 a	2120 a	1350 b	1310 b
1-Hexanal	1220 a	690 c	940 b	430	370	220 b	160 b	360 a	250 a	70 b	200 a	650	870	880	700	480	460
(*E*)-2-Hexenol	290 a	240 a	140 b	130	90.0	60.0 b	90.0 a	100 a	90.0 b	30.0 c	120 a	90.0 b	180 a	160 a	170	110	160
1-Hexanol	130 a	120 a	80.0 b	60.0	80.0	4.00 b	60.0 a	70.0 a	40.0 b	40.0 b	90.0 a	70.0 b	110 a	120 a	120	100	130
(*Z*)-3-Hexenol	22.1	26.4	10.8	30.83	36.9	0.88	0.50	0.69	1.78	1.10	1.27	nd	nd	nd	nd	nd	nd
(*E*,*E*)-2,4-Heptadienal	4.15	3.98	3.07	3.87	3.26	1.16	0.99	1.02	2.34 a	1.70 ab	0.47 b	nd	nd	nd	nd	nd	nd
2-Ethyl-1-Hexanol	3.22	2.66	2.98	2.86	1.79	nd	nd	nd	nd	nd	nd	2.67	3.00	3.05	2.99	3.11	2.93
2-Octanone	2.66	2.43	2.45	2.67	2.39	3.01	2.91	3.17	3.21	3.03	2.85	2.47	2.57	2.51	2.85	2.61	2.38
4-Decanol	2.38	3.13	2.24	5.02	3.84	2.37 a	1.95 b	2.1 ab	3.68 a	1.63 b	2.8 ab	4.67	3.53	4.22	6.74 a	3.8 ab	3.18 b
E,*E*-2,4-Hexadienal	1.34 a	0.82 b	1.25 a	1.94 a	0.65 b	nd	nd	nd	nd	nd	nd	1.60	0.83	0.69	1.29 a	0.49 b	0.64 b
(*Z*)-3-Hexenal	nd	nd	nd	nd	nd	25.1 a	8.67 b	14.3 b	nd	nd	nd	nd	nd	nd	nd	nd	nd
(*E*)-3-Hexenol	nd	nd	nd	nd	nd	67.8 a	75.3 a	41.3 b	56.5	50.5	52.7	125 a	63.0 b	29.4 b	111	45.0	41.0
1-Octene-3-ol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.20	0.17	nd	nd	0.79 a	0.61 b
Decanal	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.62	0.73	0.63	2.18	0.95	0.79
5-Ethyl 2-heptanol	nd	nd	nd	nd	nd	2.38	1.07	1.90	6.35 a	0.92 c	3.03 b	nd	nd	nd	nd	nd	nd
SUM	*5980 a*	*3520 b*	*3860 b*	*1900*	*1780*	*1150 b*	*1040 b*	*1710 a*	*1440 a*	*410 b*	*1230 a*	*2100 b*	*3650 a*	*3170 a*	*3230*	*2090*	*2120*
Alcohols (µg/L)																	
(*Z*)-2-Pentenol	14.5	12.7	13.6	12.2	10.8	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
(*E*)-2-Pentenol	nd	nd	nd	nd	nd	2.85	2.08	2.90	5.06	4.40	4.87	6.89	5.68	7.01	10.6 a	7.52 b	6.47 b
2-Methyl 1-Butanol	nd	nd	nd	nd	nd	2.06	2.24	3.41	2.93 a	2.69 a	20.3 b	0.37	1.62	0.56	32 a	3.70 b	3.81 b
3-Methyl 1-Butanol	nd	nd	nd	nd	nd	2.90 b	3.14 b	4.54 a	4.78 b	6.00 b	41.3 a	0.68	1.68	1.22	40.8 a	5.55 b	6.25 b
3-Methyl 3-Buten-1-ol	nd	nd	nd	nd	nd	4.73	4.08	4.79	4.05	3.45	4.65	nd	nd	nd	nd	nd	nd

SUM	*14.5*	*12.7*	*13.6*	*12.2*	*10.8*	*12.5*	*11.5*	*15.7*	*16.8 b*	*16.6 b*	*71.2 a*	*7.94*	*8.98*	*8.78*	*83.5 a*	*16.8 b*	*16.5 b*
Fatty acids(µg/L)																	
Octanoic acid	3.91	3.30	3.72	3.63	4.10	nd	2.22	1.59	6.16	5.53	5.70	3.18	2.80	2.65	6.55 a	3.01 b	3.26 b
Hexanoic acid	11.7	11.0	11.2	12.9	9.03	4.43	4.91	4.86	7.94	8.31	12.7	8.72 b	10.7 a	8.30 b	19.3	17.4	9.24
SUM	15.6	14.3	15.0	16.6	13.1	4.43	7.13	6.45	14.1	13.8	18.4	11.9 b	13.5 a	10.9 b	25.9	20.5	12.5
Fatty acid esters(µg/L)																	
Octyl butyrate	6.23	6.12	6.93	5.34	4.88	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
2-Pentyl propionate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	17.5	14.0	12.6	26.1 a	8.48 b	11.0 ab
SUM	*6.23*	*6.12*	*6.93*	*5.34*	*4.88*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*17.5*	*14.0*	*12.6*	*26.1 a*	*8.48 b*	*11.0ab*
Volatile Phenols and benzene derivatives (µg/L)																	
2-Phenyl ethanol	nd	nd	nd	nd	nd	100	110	110	250	220	150	30.0	40.0	30.0	220 a	30.0 b	30.0 b
Benzeneacetaldehyde	7.94 b	11.0 ab	17.4 a	11.9 b	23.3 a	8.26 b	17.8 b	34.0 a	5.26 b	8.69 b	17.5 a	8.33 b	9.92 b	20.8 a	10.91	11.27	11.54
2-Phenoxy ethanol	7.16	5.36	8.70	7.19	8.24	nd	nd	nd	nd	nd	nd	6.08 a	3.78 b	3.67 b	12.52	8.80	8.10
Benzyl Alcohol	5.89	4.21	6.44	6.37 b	8.59 a	27.8	28.2	29.6	24.9 a	19.6 b	26.7 b	7.02	6.47	6.32	23.6 a	8.49 b	7.78 b
Vanillin	3.83	3.73	5.62	4.92 a	3.34 b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Benzophenone	2.76	2.85	2.84	3.10	2.63	2.52 b	3.28 a	2.14 b	5.10	3.97	3.61	2.30 ab	1.99 b	2.66 a	4.68	2.70	2.90
Benzaldehyde	1.13	0.87	1.11	1.70	2.04	1.35	1.6	1.16	1.94	2.13	1.30	1.16	0.76	0.86	2.28	1.43	1.22
Vanillin, acetate	nd	nd	nd	nd	nd	3.17	2.05	2.07	2.16	2.01	4.16	nd	nd	nd	nd	nd	nd
3-Hydroxy-4-methoxy benzaldehyde	nd	nd	nd	nd	nd	3.36	3.69	1.96	4.8 ab	4.23 b	6.28 a	8.32 a	2.93 b	2.43 b	8.21	4.10	4.20
SUM	*28.0*	*28.0*	*42.0*	*35.0*	*48.0*	*140*	*160*	*180*	*290*	*257*	*208*	*67.0*	*70.0*	*69.0*	*280 a*	*60.0 b*	*60.0 b*
Monoterpenes (µg/L)																	
Linalool	nd	nd	nd	nd	nd	3.37	2.19	4.09	24.5 b	91.3 a	43.7 b	nd	2.69	2.29	nd	3.56	3.22
(*E*)-Linalool oxide	nd	nd	nd	nd	nd	1.06	0.80	1.66	2.92 b	7.84 a	4.50 b	nd	nd	nd	nd	nd	nd
3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)	nd	nd	nd	nd	nd	2.49	2.52	2.43	2.70	3.75	3.50	nd	nd	nd	nd	nd	nd
Geraniol	nd	nd	nd	nd	nd	2.17 a	1.47 b	2.0 ab	4.17 b	3.72 b	12.0 a	nd	nd	nd	nd	nd	nd
2,6-Dimethyl 2,7-Octadiene-1,6-diol	nd	nd	nd	nd	nd	3.60 a	2.72 b	2.17 b	57.1 b	83.0 a	54.9 b	nd	9.80	8.69	8.44	5.99	8.49

(*E*)-3,7-Dimethyl-, 2,6-Octadien-1-ol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	3.70 b	12.4 a	13.7 a	8.20	4.30	8.70
2,6-Dimethyl 3,7-Octadiene-2,6-diol	nd	nd	nd	nd	nd	9.76	8.46	8.97	44.7 b	112 a	65.3 b	nd	nd	nd	nd	nd	nd
2,6-Dimethyl 1,7-Octadiene-3,6-diol	nd	nd	nd	nd	nd	nd	nd	nd	6.90 b	22.2 b	29.2 a	nd	nd	nd	nd	nd	nd
2,2-Dimethyl 4-Octen-3-ol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.32	1.06	2.06 a	0.97 b	1.4 ab
SUM	*nd*	*nd*	*nd*	*nd*	*nd*	*22.5*	*18.2*	*21.4*	*143 c*	*324 a*	*213 b*	*3.70 b*	*26.2 a*	*25.8 a*	*18.7*	*14.8*	*21.9*
SUM of all free volatile compounds **(µg/L)**	6040 a	3580 b	3940 b	1970	1860	1340 b	1240 b	1940 a	1910 a	1020 b	1740 a	2210 b	3790 a	3310 a	3670	2220	2250

[a] The first, second and last harvest dates represents EL-37, EL-38 and EL-39 stages corresponding to 918, 928, and 935 GDD in 2019 and to 1034, 1083 and 1132 GDD in 2020.

[b] Each value represents the means of three field replicates (n=3). For a given variety and year, values followed by a different letter are significantly different at P≤0.05 according to Turkey's honestly significant difference test (Please refer to Supplemental material for interactions statistics and p values).

2.5.4 Bound volatile composition of grapes

Seven classes of bound volatile compounds were characterized in the berries of studied interspecific hybrid varieties, namely: (1) fatty acid degradation products (FADP), (2) alcohols, (3) fatty acids (FA), (4) monoterpenes (MT), (5) volatile phenols and benzene derivatives, (6) C_{13}-norisoprenoids and (7) other volatile compounds (Table 2.7). Among them, volatile phenols and benzene derivatives accounted for the largest proportion of the bound volatile compounds (51 %) in L'Acadie blanc berries. In comparison, the monoterpenes accounted for the largest proportion of bound volatile compounds in both Osceola Muscat (59 %) and Seyval blanc (49 %) at HD2 and HD3. Within volatile phenols and benzene derivatives, benzyl alcohol and 2-phenyl ethanol were the major compounds in the studied varieties.

The total concentration of FADP did not show any distinct pattern with ripening, except in Seyval blanc berries harvested in 2020. These berries showed a significant increase from *99.1 to 128* µg/L with ripening (Table 2.7). The total monoterpene content increased significantly with ripening in L'Acadie blanc (0.32 to 0.55 in 2019, 0.25 to 0.39 mg/L in 2020), Osceola Muscat (0.8 to 3.52 mg/L in 2020) and Seyval blanc (0.58 to 1.44 in 2019, 0.83 to 1.46 mg/L in 2020) (Table 2.7). MTs such as (*Z*)-linalool oxide, linalool, geraniol and neric acid did not accumulate in Osceola Muscat berries; in contrast, the accumulation of MTs such as (*Z)*-8-hydroxylinalool, 3,7-dimethyl-1,6-octadien-3-ol and 2,6-Dimehtyl-1,7-octadiene-3-ol accounted for the highest proportion of MTs in this variety. The C_{13}-norisoprenoid compounds such as 3-oxo-*α*-ionol, *β*-ionol, 3-hydroxy-5,6-epoxy-*β*-ionone and dihydro-3-oxo-*β*-ionol were only detected in L'Acadie blanc berries and were the main C_{13} compounds detected in this variety with 3-hydroxy-*β*-damascenone. In both years, furaneol significantly increased with ripening in Osceola Muscat berries. However, it was not detected in the other grape varieties.

Table 2.7 Bound volatile compounds from the juice of the interspecific hybrid grapes L'Acadie blanc, Osceola Muscat and Seyval blanc harvested in the Province of Nova Scotia (Canada) at three dates in 2019 and 2020.

	L'Acadie blanc					**Osceola Muscat**						**Seyval blanc**					
Year	2019			2020		2019			2020			2019			2020		
Harvest date	3rd Oct[a]	10th Oct	22nd Oct	15th Sep	28th Sep	3rd Oct	10th Oct	22nd Oct	15th Sep	28th Sep	8th Oct	3rd Oct	10th Oct	22nd Oct	15th Sep	28th Sep	8th Oct
GDD	918	928	935	1034	1083	918	928	935	1034	1083	1132	918	928	935	1034	1083	1132
Fatty acid degradation products (µg/L)																	
1-Hexanol	39.7[b] b	42.2 ab	45.9 a	35.0 b	52.2 a	8.50	8.80	7.34	12.4	16.3	20.1	23.4 b	25.5 b	35.5 a	28.3 b	41.1 a	40.9 a
(*E*)-2-Hexenal	35.2	32.5	35.6	90.1	88.0	8.50	7.50	5.43	32.2 a	7.90 b	20.9 ab	20.7	29.9	20.6	49.0	99.9	56.5
(*Z*) 3-Hexenol	21.3 a	19.7 ab	17.4 b	30.7	28.3	10.1 b	11.4 a	10.3b	13.8	10.4	15.8	4.70	4.48	5.90	4.30	5.16	6.02
Nonanal	17.2 a	5.08 b	7.01 ab	11.4	12.9	7.45	8.36	5.52	14.2	13.7	12.2	5.28	5.57	4.02	10.2 b	28.2 a	9.1 ab
(*E*)-2-Hexenol	8.25 a	6.29 ab	5.87 b	8.08 b	15.2 a	nd	nd	nd	nd	nd	nd	1.98 b	5.37 a	6.90 a	7.26 b	17.0 a	14.9 a
Hexanal	7.70	4.91	6.38	6.79	9.19	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
1-Pentanol	5.75 b	7.46 ab	9.91 a	7.61	10.5	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
2 Heptanol	4.39 a	3.15 b	2.69 b	4.99	4.66	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
(*Z*)-2-Hexenol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.66	nd	nd	nd	nd	nd	nd
SUM	*139*	*121*	*131*	*197*	*221*	*34.5*	*36.1*	*28.6*	*72.5*	*48.4*	*70.7*	*56.2*	*70.8*	*73.0*	*99.1b*	*191 a*	*128 ab*
Alcohols (µg/L)																	
2-Methyl 1-Butanol	24.2 b	28.4 ab	36.3 a	36.2	33.8	6.34 b	9.83 a	11.3 a	9.97	10.9	16.74	29.0	25.8	32.6	46.4	37	54.4
3-Methyl 3-Buten-1-ol	17.9 a	13.9 b	10.4 c	23.2	21.3	18.6 a	13.6 ab	10.7 b	31.6	17.0	20.0	16.1	23.5	14.3	35.1	43.4	36.6
3-Methyl 1-Butanol	14.9	13.8	13.6	20.1	18.7	11 .0	9.80	9.59	22.2	18.0	19.7	20.6	24.0	21.7	35.9	36.7	37.9
3-Methyl 2-Butenol	3.29 b	4.17 b	5.08 a	10.8	9.93	2.59 a	1.38 b	1.41 b	4.37	5.39	6.24	13.2 b	14.2 b	22.2 a	18.7	21.2	22.4
Heptan-2-ol	3.52	4.40	3.97	6.49	4.29	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
SUM	*63.9*	*64.7*	*69.4*	*96.9*	*88.1*	*38.6*	*34.6*	*33.1*	*68.2*	*52.3*	*63.0*	*78.8*	*87.5*	*90.9*	*136*	*138*	*151*
Fatty acids (µg/L)																	
Hexanoic acid	4.24 ab	5.02 a	3.58 b	5.08	7.08	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Monoterpenes (µg/L)																	
(*E*) 8-Hydroxylinalool	217 c	317 b	417 a	149	251	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
(Z)-Linalool oxide	32.6 b	30.9 b	39.5 a	19.2 b	36.0 a	nd	nd	nd	nd	nd	nd	30.6	26.5	25.0	31.2	34.6	35.2
(*E*)-Linalool oxide	26.8	24.1	22.1	12.1	12.0	11.2	9.58	9.08	19.4 b	31.6 b	84.9 a	17.4	15.0	14.4	15.8	14.7	13.6
Hotrienol	10.8 b	14.9 b	25.4 a	6.82 b	29.9 a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Linalool	7.16 b	7.48 b	13.3 a	5.40 b	14.0 a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Nerol	3.72 c	11.7 b	14.5 a	3.66 b	8.77 a	nd	nd	nd	18.3	26.5	30.9	6.03 b	14.0 b	39.1 a	14.1	19.4	19.8
3,7-Dimethyl 1,6-Octadien-3-ol	nd	nd	nd	nd	nd	6.52	6.25	8.27	18.2 b	59 ab	102 a	4.97 b	7.12 b	18.9 a	5.99 b	16.4 a	22.8 a
L-α-Terpineol	nd	nd	nd	nd	nd	nd	nd	nd	nd	8.86	15.0	nd	nd	nd	nd	nd	nd
(*Z*)-8-Hydroxylinalool	nd	nd	nd	nd	nd	360	320	480	520 b	1050b	2690 a	200 b	700ab	950 a	390 b	600ab	950 a
Isoborneol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	3.16 a	2.39 b	1.79 c	2.57	4.10	3.68
Geraniol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	47.6 b	70.3 b	153 a	75.9 b	161 a	137 a
Neric acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	20.7 a	16.9 a	3.72 b	10.5	21.2	26.2
2,6-Dimethyl 1,7-Octadien-3,6-diol	nd	nd	nd	nd	nd	nd	nd	nd	nd	11.5	27.1	nd	nd	nd	nd	nd	nd
2,6-Dimehtyl-1,7-octadiene-3-ol	nd	nd	nd	nd	nd	26.3	31.4	52.9	33.6 b	108 b	319 a	4.81 b	11.0 ab	20.6 a	11.20	5.08	8.76
Lilac alcohol C	3.32 b	3.98 b	6.06 a	1.57 b	6.51 a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Lilac alcohol B	nd	nd	nd	nd	nd	13.1	11.2	15.1	16.1 b	24.0 ab	36.0 a	nd	nd	nd	nd	nd	nd
3-Buten-2-ol, 4-2,6,6-trimethyl-2-cyclohexen-1-yl	22.2	17.6	12.7	47	33.5	20.3	22.5	25.8	nd	7.45 b	23.5 a	nd	nd	nd	nd	nd	nd
2-Butanone, 4-(2,6,6-trimethyl-2-cyclohexen-1-yl)(R)	nd	nd	nd	nd	nd	59.1	56.3	50.8	27.5	25.5	36.6	29.2 b	20.2 c	33.5 a	33.6	36.1	39.1
2-Cyclohexen-1-one, 4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl	nd	nd	nd	nd	nd	151 b	155 b	181 a	131	114	139	43.8	35.1	43.2	60.9	47.1	49.0
2-Cyclohexen-1-one, 3-(3-hydroxybutyl)-2,4,4-trimethyl	nd	nd	nd	nd	nd	8.42	8.44	8.38	6.10 b	7.8 ab	10.2 a	8.18	7.56	9.50	15.8	11.0	13.0
2-Butanone, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	157 a	106 b	124 b	156	152	146.5
SUM	*320 c*	*430 b*	*550 a*	*250 b*	*390 a*	*650*	*620*	*830*	*800 b*	*1480 b*	*3520 a*	*580 b*	*1070 b*	*1440 a*	*830 b*	*1100 ab*	*1460 a*
Volatile phenols and benzene derivatives (µg/L)																	
Benzyl alcohol	700 a	650 a	570 b	770	800	160	170	160	220	190	190	480 a	320 b	490 a	620 a	470 b	500 b
2-Phenylethanol	320 a	320 a	280 b	440	390	160	160	160	280	230	230	20 0	190	180	330	220	250
Methyl vanillate	40.0 b	120 a	110 a	30.0	30.0	20.0 b	10.0 b	80.0 a	20.0 b	40.0 b	160 a	50.0 ab	40.0 b	90.0 a	50.0	40.0	50.0

m-Toluic acid, 3-tridecyl ester	30.0 a	30.0 a	20.0 b	30.0	30.0	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Eugenol	13.2	13.2	12.8	17.0	13.4	16.1	11.6	13.2	31.1	16.6	20.7	nd	nd	nd	nd	nd	nd
p-Vinylguaiacol	7.17 b	13.7 ab	16.6 a	4.97	5.28	12.4 b	28.4 a	36.3 b	6.47 b	13.9 b	52.7 a	7.40 b	9.66 b	27.7 a	12.37	5.59	7.73
6-Methoxy eugenol	4.91	5.85	5.74	5.38	4.55	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
3-Hydroxy-4-methoxy benzaldehyde	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	4.19	nd	nd	nd	nd	nd	nd
Phenol, 2-methoxy-4-(1-propenyl)	nd	nd	nd	nd	nd	22.2	26.1	19.7	14.2	19.6	18.9	nd	nd	nd	nd	nd	nd
4-Hydroxy-3-methoxy benzenemethanol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	13.0	nd	nd	nd	nd	nd	nd
SUM	*1110 a*	*1115 a*	*1020 b*	*1300*	*1260*	*390 b*	*410 b*	*460 a*	*560*	*510*	*680*	*730 a*	*560 b*	*780 a*	*1010a*	*740 b*	*800 b*
C_{13} Norisoprenoids (µg/L)																	
3-Oxo-*α*-ionol	123 b	139 a	149 a	140	151	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
β Ionol	111 b	131 a	116 b	81.7	63.7	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
3-Oxo-7,8-dihydro-*α*-ionol	89.7	97.1	93.0	101.1	97.5	39.1 a	32.1 b	32.2 b	41.4	36.7	45.9	nd	nd	nd	nd	nd	nd
3-Hydroxy-*β*-damascone	85.5 b	91.4 ab	101.3 a	93.4	86.3	50.7 b	67.2 a	64.0 ab	52.3	50.7	49.4	79.5 a	59.0 b	88.8 a	72.3	68.3	74.9
3-Hydroxy-7,8-dihydro-*β*-ionol	29.5 b	35.2 b	47.2 a	36.9	31.5	nd	nd	nd	nd	nd	nd	31.0ab	23.4 b	36.4 a	31.0	28.6	34.0
3-Hydroxy-5,6-epoxy- *β* -ionone	16.2 a	16.2 a	13.9 b	11.8	15.8	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Dihydro-3-oxo-*β*-ionol	8.55 c	10.2 a	9.37 b	6.71	11.0	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
SUM	*460 b*	*520 a*	*530 a*	*470*	*460*	*90.0*	*100*	*100*	*9.00*	*90.0*	*100*	*110 a*	*80 b*	*130 a*	*100*	*100*	*110*
Other volatile compounds (µg/L)																	
2-Butyltetrahydrofuran,	5.32 b	6.69 a	6.04 ab	4.84	4.49	5.16 b	5.8 ab	6.26 a	5.54 b	6.24 b	10.9 a	nd	nd	nd	nd	nd	nd
Furaneol	nd	nd	nd	nd	nd	nd	1.63 b	12.6 a	1.05 b	33.5 b	243 a	nd	nd	nd	nd	nd	nd
SUM	*5.32 b*	*6.69 a*	*6.04 ab*	*4.84*	*4.49*	*5.16 b*	*7.45 b*	*18.9 a*	*20.3 b*	*39.8 b*	*254 a*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*
SUM of all bound volatile compounds **(µg/L)**	*2120 b*	*2300 a*	*2320 a*	*2320*	*2440*	*1210*	*1210*	*1470*	*1610 b*	*222 b*	*4690 a*	*1550 b*	*1870 ab*	*2510 a*	*2180*	*2290*	*2650*

[a] The first, second and last harvest dates represents EL-37, EL-38 and EL-39 stages corresponding to 918, 928, and 935 GDD in 2019 and to 1034, 1083 and 1132 GDD in 2020.

[b] Each value represents the means of three field replicates (n=3). For a given variety and year, values followed by a different letter are significantly different at P≤0.05 according to Turkey's honestly significant difference test (Please refer to Supplemental material for interactions statistics and p values).

2.5.5 Wine free volatile compounds

Volatile compound analysis of wines produced from the white interspecific hybrid grapes studied resulted in the semi-quantification of 57 compounds, including grape-derived compounds such as FADP and monoterpenes as well as fermentation derived compounds such as esters and other fermentation products (Table 2.8). Fermentation-related volatile compounds accounted for the highest proportion of wine volatile compared (98%) with variety-related volatile compounds (0.4%).

The concentration of the FADP, (*Z*)-3-hexenol, decreased with ripening for all three studied varieties. And the concentration of 1-hexanol decrease with ripening in wines from L'Acadie blanc berries harvested in 2019, while other varieties did not show any distinct pattern. The monoterpene *β*-linalool was also identified only in Osceola Muscat wines in 2020, and its concentration increased from 12.9 to 49.0 µg/L from 1034 to 1132 GDD. The phenolic ester phenyl ethyl acetate was detected at very low levels in wine. Its concentration increased in 2020 by 172%, 126% and 166% with ripening in L'Acadie blanc, Osceola Muscat and Seyval blanc, respectively. In 2019, an increase was only observed in L'Acadie blanc. Fermentation alcohol 3-methyl-1-butanol was the main aroma compound found in wine from all analyzed cultivars (70%), followed by 2-phenylethanol. Concentration of 2-phenylethanol showed a significant increase from 1034 to 1132 GDD in L'Acadie blanc and Osceola Muscat wines from 2020. A similar trend was observed for isophenyl acetate concentration of Osceola Muscat wines in 2020. Furaneol was only observed in Osceola Muscat wines in 2020, and its concentrations in wine increased with the berry ripening (6.9 to 66.6 µg/L).

Table 2.8 Free volatile compounds from the wines of the interspecific hybrid grapes L'Acadie blanc, Osceola Muscat and Seyval blanc harvested in the Province of Nova Scotia (Canada) at three dates in 2019 and 2020.

	Acadie blanc						**Osceola Muscat**						**Seyval blanc**					
Year	2019			2020			2019			2020			2019			2020		
Harvest date	3rd Oct[a]	10th Oct	22nd Oct	15th Sep	28th Sep	8th Oct	3rd Oct	10th Oct	22nd Oct	15th Sep	28th Sep	8th Oct	3rd Oct	10th Oct	22nd Oct	15th Sep	28th Sep	8th Oct
GDD	918	928	935	1034	1083	1132	918	928	935	1034	1083	1132	918	928	935	1034	1083	1132
Fatty acid degradation products (µg/L)																		
(*Z*)-3-Hexenol	132[b] a	112 a	75.0 b	122 a	64.9 b	87 ab	464 a	330 b	229 b	870 a	362 b	312 b	178 a	99.5 b	92.9 b	109.7	72.9	56.9
1-Hexanol	1370 a	1300 a	960 b	1750	1420	1450	270	270	200	500 b	600 ab	760 a	760	600	680	1250	1070	1020
SUM	*1510 a*	*1410 a*	*1040 b*	*1870*	*1490*	*1540*	*740 a*	*600 a*	*430 b*	*1370*	*960*	*1070*	*940*	*700*	*78 0*	*1360*	*1150*	*1080*
Fatty acid ethyl esters (µg/L)																		
Diethyl butanedioate	790	910	770	320	380	400	980	980	930	490	450	560	1270	1100	1110	390	360	370
Ethyl octanoate	650	590	580	560	680	740	330	270	270	780	930	510	320	830	410	640	660	680
Diethyle 2-hydroxypentanedioate	550	610	580	300 ab	420 a	250 b	360 b	480 a	510a	230	260	230	500	500	610	350	420	360
Ethyl hexanoate	250	220	210	200 ab	290 a	170 b	190 a	160 ab	120 b	150	190	150	150	150	150	200	240	200
Ethyl 2-oxopropanoate	210	180	150	150 a	60.0 b	80.0 b	330	230	230	nd	nd	nd	220	140	170	100	130	110
Ethyl butanoate	130	120	120	100	140	130	90.0	70.0	70.0	80.0	110	80.0	90.0	120	90.0	110	110	80.0
Ethyl 2-hydroxy-3-	90.0	110	90.0	110	80.0	110	90.0	90.0	80.0	120	90.0	160	110	100	110	110	110	150

phenylpropanoate																		
Ethyl 4-hydroxybutanoate	60.0	80.0	90.0	200	280	340	40.0 b	50.0 a	70.0 a	300	500	880	70.0	60.0	100	250	330	620
Ethyl 2-methylprpanoate	68.3	61.7	53.2	53.8	44.9	70.5	65.8	51.5	55.7	55.1 b	48.6 b	99.1 a	76.9	76.1	60.6	61.9	66.8	89.7
Ethyl propanoate	64.5	69.9	71.3	49.8	64.8	140.6	63.2	51.0	62.6	84.6	81.5	82.4	71.7 a	52.6 b	84.9 a	63.2	69.6	66.9
Ethyl 2-hydroxy-4-methylpentanoate	47.1	59.4	60.1	56 ab	137 a	48.2 b	58.8	63.1	66.0	117	173	181	61.5	53.4	58.4	66.3	74.7	115
Ethyl 3-methylbutanoate	40.8	41.2	34.0	56.3	49.2	53.8	29.5	23.5	19.8	109 a	58 b	71.8 b	38.9	40.6	39.9	55.6	41.7	40.1
Ethyl decanoate	34.0	27.0	44.9	26.9 b	47.6 a	34 ab	23.1	20.7	16.5	20.1	28.5	18.4	11.0	16.0	9.15	38.6	48.9	46.1
Ethyl 3-hydroxybutanoate	33.6 b	46.5 a	52.1 a	43.1 b	82.5 a	61 ab	29.0	38.4	35.2	37.9	46.6	46.8	36.8	40.4	50.1	35.1 b	53 ab	70.7a
Ethyl 3-methyl butyl butanedioate	23.7	16.9	22.6	6.71	7.07	10.4	25.2	26.4	27.7	7.45	8.63	11.8	34.4	32.7	52.1	14.8	8.50	14.3
Ethyl 3-hydroxypropionate	19.4	22.5	23.6	11.0	12.1	9.38	24.4 a	17.1 b	18 ab	37.8	23.6	55.2	44.2	21	41.9	12.5	12.7	9.48
Ethyl 2-methylbutanoate	7.58	8.50	7.22	4.50 ab	4.01 b	6.45 a	8.12	6.53	7.30	5.7 ab	5.26 b	9.36 a	10.08	7.11	10.79	8.76	6.59	7.12
SUM	*3080*	*3170*	*2970*	*2330*	*2790*	*2670*	*2720*	*2630*	*2590*	*2610*	*3020*	*3140*	*3120*	*3350*	*3160*	*2520*	*2750*	*3030*
Fatty acids (µg/L)																		
2,4-Hexadienoic acid	4890	4830	4460	nd	nd	nd	4870	5010	4730	nd	nd	nd	5340	2540	6420	nd	nd	nd
Octanoic acid	2200	2190	2510	580	540	720	1270	1700	1460	440	470	630	1650	1710	1440	580	530	580

Hexanoic acid	920	960	960	340 b	640 a	340 b	390	530	490	410	430	340	740	650	700	408	360	400
Isobutyric acid	380	320	300	320	250	390	390	330	340	310 b	380 ab	580 a	490	340	600	350	360	560
Heptanoic acid	120	120	100	40.0	30.0	40.0	60.0	50.0	50.0	60.0	50.0	60.0	100	100	120	100	40.0	40.0
3-Methylbutanoic acid	140	140	140	230	200	190	150	150	150	170 b	190 ab	300 a	200 ab	90 b	300 a	210	240	240
9 Decenoic acid	110	60.0	50.0	5 .0	4.0	12.0	90.0 a	50.0 b	60.0 b	50.0	50.0	80.0	110	70.0	160	10.0	10.0	10.0
2-Methylbutanoic acid	70 .0	70.0	120	50.0	80.0	170	70.0	60.0	70.0	40.0 b	90.0 b	230 a	60.0	50.0	180	200	90.0	190
Butanoic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	150 a	140 b	140 b	nd	nd	nd	nd	nd	nd
SUM	*8820*	*8696*	*8640*	*1560*	*1730*	*1860*	*7290*	*7870*	*7360*	*1620*	*1790*	*2370*	*8660*	*5550*	*6590*	*1890*	*1640*	*2020*
Monoterpenes (µg/L)																		
β-Linalool	nd	nd	nd	nd	nd	nd	nd	nd	nd	12.9 c	34.0 b	49.0 a	nd	nd	nd	nd	nd	nd
Phenolic esters (µg/L)																		
Phenethyl acetate	16.3 ab	26.0 ab	41.3 a	80.0 b	96.9 b	220 a	32.9	45.4	51.3	57.9 b	82.0 ab	131 a	43.0	39.4	37.4	74.7 b	126 a	199 a
Other fermentation volatile compounds (µg/L)																		
3-Methylbutanol (mg/L)	90.3	98.0	93.3	127	108	160	77.9	82.0	82.1	104 b	108 b	170 a	102	111	124	145	146	188
2-Phenylethanol (mg/L)	22.2	24.7	24.4	39.7 b	28.6 b	77.4 a	28.6 b	31.4 ab	39.2 a	42.1 b	37.5 ab	76.2 a	27.9	26.0	43.1	51.9	45.4	69.5
Diethyl malate	4150 a	4230 a	2890 b	1090a	790 b	500 c	5202 a	4800 ab	4180 b	1790 a	1170 ab	1040 b	7480 a	6300ab	5220 b	2150	1230	800
Ethyl lactate	1840	2300	1950	1450	1590	1620	1550	1720	1800	1840	1590	1720	2080	1810	2510	1470	1650	1610
γ-Butyrolactone	580	540	520	400 ab	450 a	330 b	380	440	480	300	380	430	480	540	690	460	490	480
Tartaric acid	260 a	220 a	160 b	50.0	30.0	30.0	420 a	360 ab	290 b	110	70.0	70.0	460	320	390	90.0	50.0	30.0

diethyl ester																		
2 Ethyl hexanol	220	240	580	210	260	140	100 b	210 b	410 a	nd	nd	nd	540	210	100	200	200	90.0
Isophenyl acetate	80.0	90.0	140	380	560	720	70.0	80.0	120	220 b	260 b	440 a	130	100	190	300	570	790
α-Butyrolactone	90	110	120	170	150	100	110	100	140	230	230	340	120	90.0	110	150	140	180
Diethyl succinate	80.0 b	90.0 a	100 a	80.0 b	120 a	110 a	60.0 b	80.0 a	80.0 a	80.0	100	100	60.0	110	120	70.0	100	90.0
Pantolactone	70.0	60.0	50.0	100	70.0	80.0	60.0	50.0	20.0	60.0	60.0	100	100	60.0	50.0	130	100	100
3-Methyl pentanol	50.0 c	70.0 b	100 ca	90.0 c	140 b	200 a	30.0 b	30.0 b	50.0 a	50.0 b	70.0 b	110 a	50.0 b	50.0 b	80.0 a	80.0	120	150
2-Tert-butyl-5-propyl-1,3-dioxolan-4-one	10.0	10.0	10.0	nd	nd	nd	nd	nd	nd	110 a	70.0 b	80.0 ab	10.0	10.0	10.0	10.0	10.0	10.0
3,4-Dimethyl 2-hexanol	61.7	70.5	58.6	11.7 b	15.0 ab	27.1 a	14.8	16.1	16.9	15.2	14.6	15.0.	64.6	53.0	78.9	12.1	13.7	18.7
4-Methyl 1-pentanol	40.1	47.4	40.9	44.5 b	42.0 b	79.3 a	15.4	21.5	26.4	42.8 ab	29.4 b	58.8 a	42.8	40.5	53.1	52.6	55.9	74.2
2-Ethylbutanol	29.5	26.9	21.8	62.6	55.4	76.4	14.1	15.6	15.3	79.9	81.7	86.1	22.8	46.9	34.2	70.6	77	68.8
2,6 Di (T-Butyl)-4-hydroxy-4-methyl-2,5 cyclohexadien-1-one	19.1	14.6	17.4	16.5	18.0	24.1	26.8	28.7	21.6	29.4	21.2	23.0	28.4	18.9	30.8	24	21.3	23.9
Ethyl 2-hydroxyisovalerate	4.01	3.86	5.82	4.84	4.60	8.43	2.67 a	4.9 ab	6.02 b	5.70 b	4.15 b	9.02 a	3.86	3.83	6.10	7.47	4.59	7.82
Furaneol	nd	nd	nd	nd	nd	nd	nd	nd	nd	6.91 c	38.7 b	66.6 a	nd	nd	nd	nd	nd	nd

SUM (mg/L)	*120*	*131*	*124*		*171ab*	*141 b*	*242 a*		*115*	*121*	*129*		*151 b*	*150 b*	*251 a*		*141*	*146*	*177*		*202*	*196*	*262*
SUM of all wine volatiles (mg/L)	134	144	137		177 b	147 ab	248 a		125	133	139		157 b	156 b	258 a		154	156	191		208	202	269

[a] The first, second and last harvest dates represents EL-37, EL-38 and EL-39 stages corresponding to 918, 928, and 935 GDD in 2019 and to 1034, 1083 and 1132 GDD in 2020.

[b] Each value represents the means of three field replicates (n=3). For a given variety and year, values followed by a different letter are significantly different at $P \leq 0.05$ according to Turkey's honestly significant difference test (Please refer to Supplemental material for interactions statistics and p values).

2.5.6 Principal component analysis for wine volatile

Principal component analysis (PCA) of wine volatile compounds of L'Acadie blanc, Osceola Muscat and Seyval blanc showed different ripening patterns and seasonal variations between 2019 and 2020 (Figures 2.1, 2.2 and 2.3). There was a clear separation of the variables in PC 1 for year of harvest, whereas harvesting dates (HD1, HD2 and HD3) were separated along PC 2 in all three studied cultivars.

Phenyl ethyl acetate and isophenyl acetate mostly accumulated at latest harvesting stage (HD3) in 2020 in both cultivars L'Acadie blanc (quadrants 2, figure 2.2) and Seyval blanc (quadrants 2 and 4 figure 2.4). On the other hand, FADP such as (*Z*)-3 hexenol and 1-hexanol were found in higher concentrations at the initial stages of maturity (HD1) in both the varieties L'Acadie blanc (quadrants 1 and 4, Figure 2.2) and Seyval blanc wines (quadrants 1 and 2, Figure 2.4) and the concentrations significantly decreased with ripening. Monoterpenes such as *β*-linalool and other fermentation compounds such as furaneol only accumulated in Osceola Muscat wines made from berries harvested at HD3 (later maturity) and in year 2020 (quadrant 2, Figure 2.3).

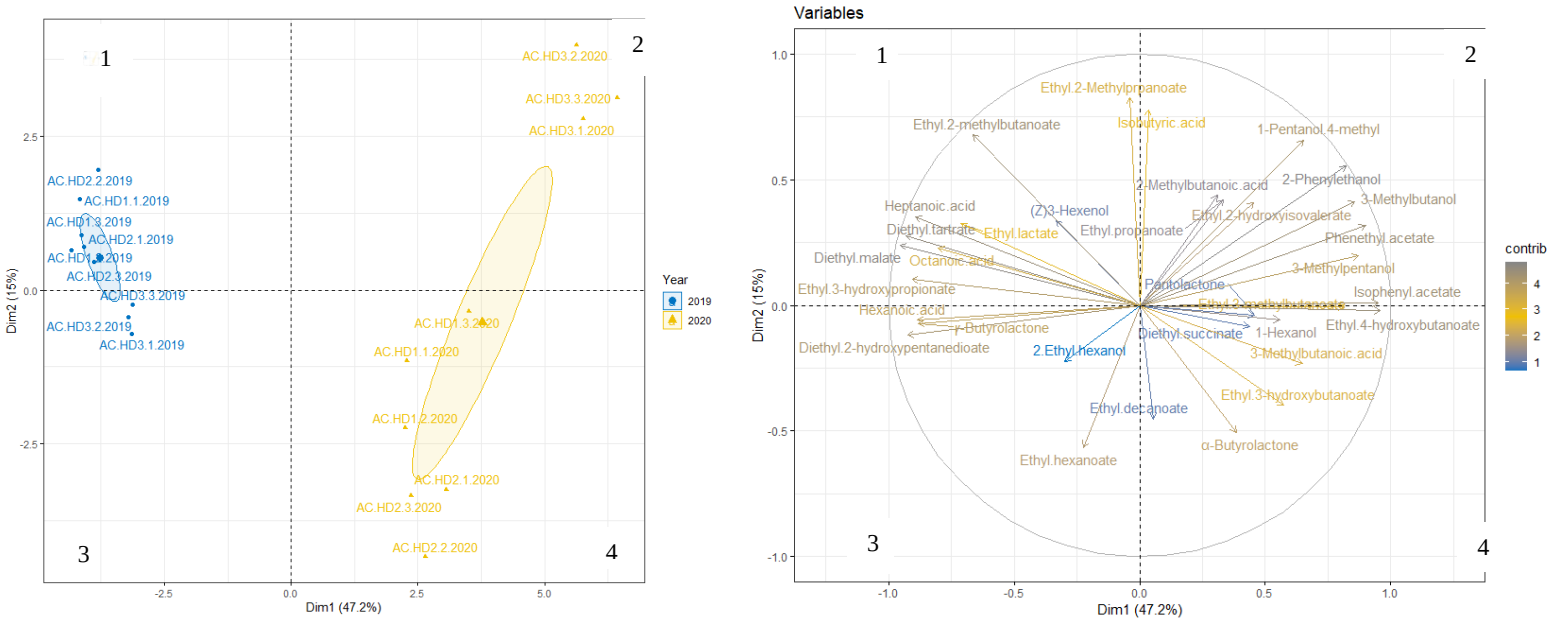


Figure 2.2 Principal component analysis (PCA) for wine-free volatile compounds (33 compounds) of L'Acadie blanc harvested at three harvesting dates HD1, HD2 and HD3. HD1, HD2 and HD3 represents EL-37, EL-38 and EL-39 stages corresponding to 918, 928, 935 growing-degree days (GDD) in 2019 and 1034, 1083 and 1132 GDD in 2020 in Nova Scotia. Samples (n=18) are plotted on the left graphs whereas variables are plots on the right graphs. Variables are (*Z*)-3-Hexenol , 1-Hexanol, Ethyl propanoate , Ethyl 2-Methylpropanoate, Ethyl 2-methylbutanoate, Ethyl 3-methylbutanoate, Ethyl 3-hydroxypropionate, Ethyl 3-hydroxybutanoate, Ethyl hexanoate, Ethyl 4-hydroxybutanoate, Diethyl 2-hydroxypentanedioate, Ethyl decanoate, Ethyl lactate, Isophenyl acetate, α-Butyrolactone, Pantolactone, 2-Phenylethanol, γ-Butyrolactone, Diethyl tartrate, 3-Methylbutanol, 4-Methyl-pentanol, 3-Methylpentanol, 2-Ethyl hexanol, Ethyl 2-hydroxyisovalerate, Diethyl malate, Diethyl succinate, Heptanoic acid, Hexanoic acid, Octanoic acid, Isobutyric acid, 3-Methylbutanoic acid, 2-Methylbutanoic acid and Phenethyl acetate (colour scale from blue-yellow –ash represent the contribution from each parameter towards the model (1 weak contribution and 4 strong contribution) and each point in the left plot represent the combination of cultivar, harvesting date, replicate and year).

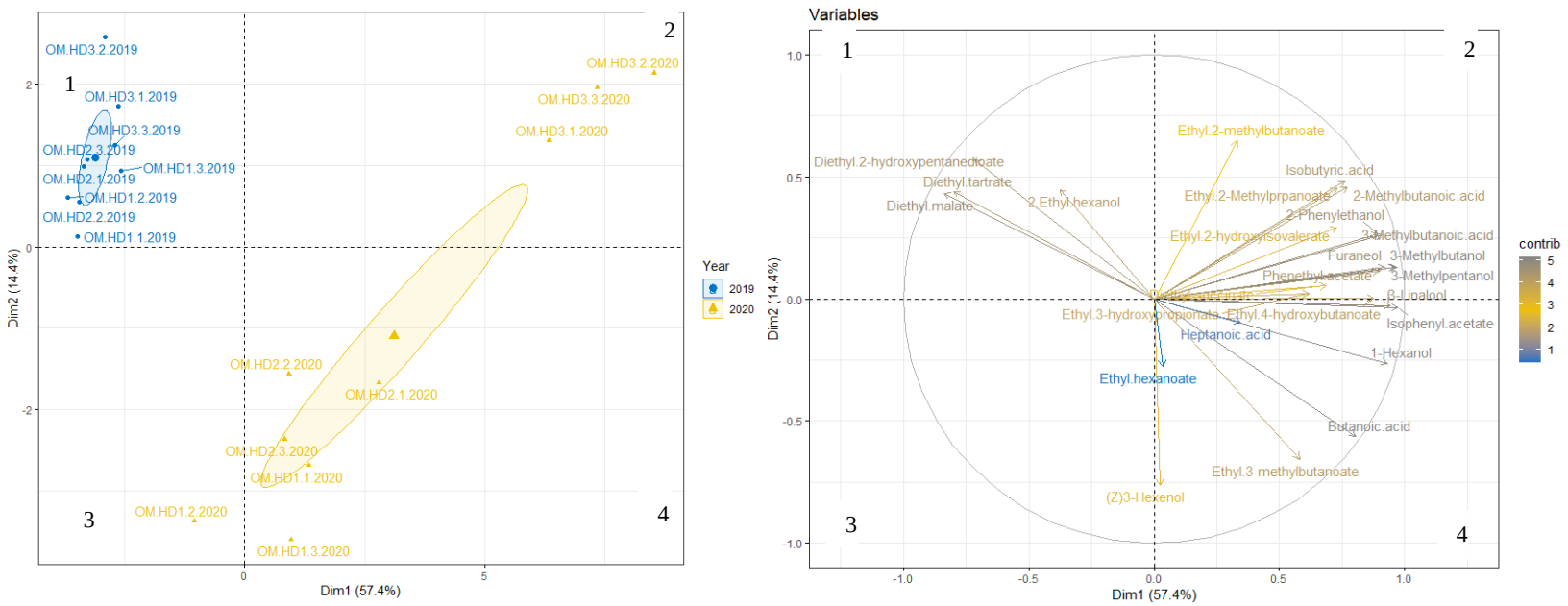


Figure 2.3 Principal component analysis (PCA) for wine-free volatile compounds (26 compounds) of Osceola Muscat harvested at three harvesting dates HD1, HD2 and HD3. HD1, HD2 and HD3 represents EL-37, EL-38 and EL-39 stages corresponding to 918, 928, 935 growing-degree days (GDD) in 2019 and 1034, 1083 and 1132 GDD in 2020 in Nova Scotia. Samples (n=18) are plotted on the left graphs whereas variables are plots on the right graphs. Identified 26 variables are (Z)3-Hexenol , 1-Hexanol, Ethyl 2-Methylpropanoate, Ethyl 2-methylbutanoate, Ethyl 3-methylbutanoate, Ethyl 3-hydroxypropionate, Ethyl hexanoate, Ethyl 4-hydroxybutanoate, Diethyl 2-hydroxypentanedioate, Isophenyl acetate, 2-Phenylethanol, Diethyl tartrate, 3-Methylbutanol, 3-Methylpentanol, 2-Ethyl hexanol, Ethyl 2-hydroxyisovalerate, Diethyl malate, Diethyl succinate, Furaneol, Heptanoic acid, Isobutyric acid, Butanoic acid, 3-Methylbutanoic acid, 2-Methylbutanoic acid, β-Linalool and Phenethyl acetate (colour scale from blue-yellow –ash represents the contribution from each parameter towards the model (1 weak contribution and 4 strong contribution) and each point in the left plot represent the combination of cultivars, harvesting date, replicate and year).

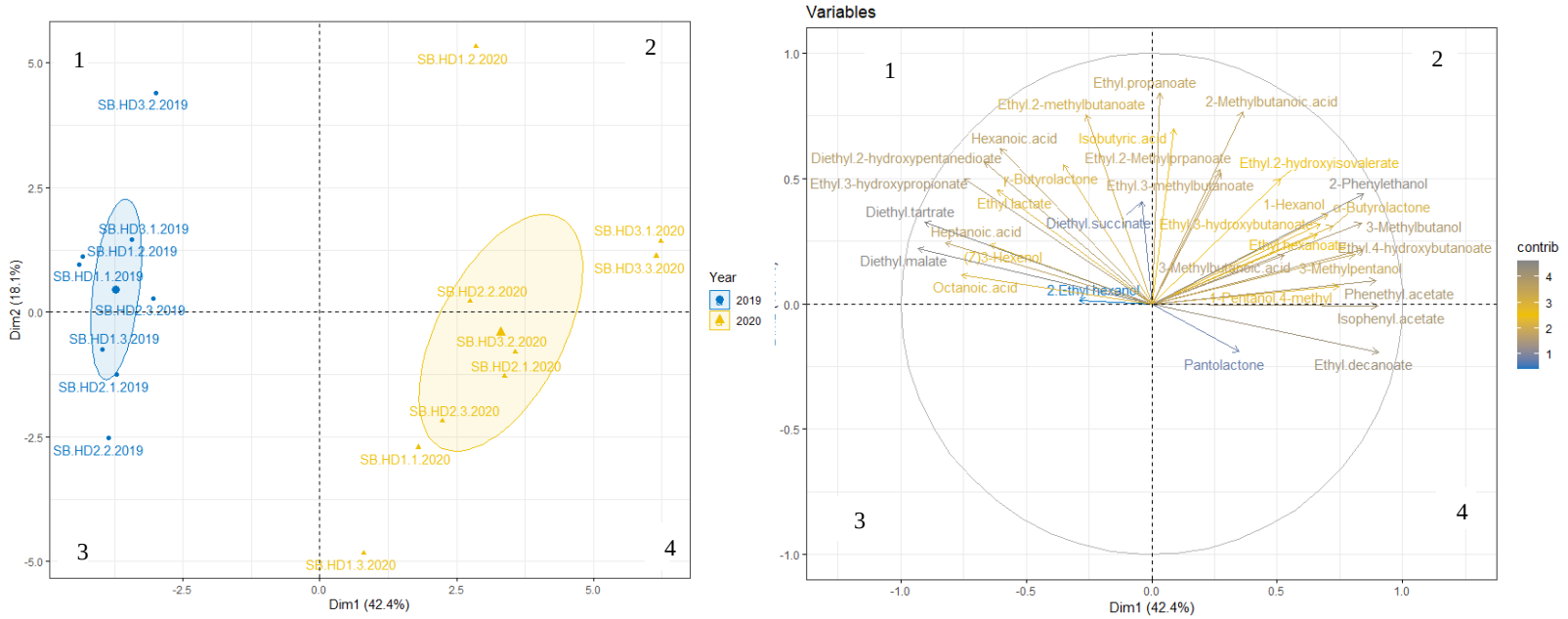


Figure 2.4 Principal component analysis (PCA) for wine-free volatile compounds (33 compounds) of Seyval blanc harvested at three harvesting dates HD1, HD2 and HD3. HD1, HD2 and HD3 represents EL-37, EL-38 and EL-39 stages corresponding to 918, 928, 935 growing-degree days (GDD) in 2019 and 1034, 1083 and 1132 GDD in 2020 in Nova Scotia. Samples (n=17) are plotted on the left graphs whereas variables are plots on the right graphs. Variables are (Z)3-Hexenol , 1-Hexanol, Ethyl propanoate , Ethyl 2-Methylpropanoate, Ethyl 2-methylbutanoate, Ethyl 3-methylbutanoate, Ethyl 3-hydroxypropionate, Ethyl 3-hydroxybutanoate, Ethyl hexanoate, Ethyl 4-hydroxybutanoate, Diethyle 2-hydroxypentanedioate, Ethyl decanoate, Ethyl lactate, Isophenyl acetate, α-Butyrolactone, Pantolactone, 2-Phenylethanol, γ-Butyrolactone, Diethyl tartrate, 3-Methylbutanol, 1-Pentanol 4-methyl, 3-Methylpentanol, 2 Ethylhexanol, Ethyl 2-hydroxyisovalerate, Diethyl malate, Diethyl succinate, Heptanoic acid, Hexanoic acid, Octanoic acid, Isobutyric acid, 3-Methylbutanoic acid, 2-Methylbutanoic acid and Phenethyl acetate (colour scale from blue-yellow –ash represent the contribution from each parameter towards the model (1 weak contribution and 4 strong contribution) and each point in the left plot represent the combination of cultivar, harvesting date, replicate and year).

2.6 Discussion

2.6.1 Chemical changes during ripening

The accurate assessment of grape maturity and the determination of the optimal harvesting date are essential for producing quality wines. The most common markers for berry maturity assesment are total soluble solid (TSS), and titratable acidity (TA), and their values largely depend on the grape variety, growing conditions and berry ripening (Gao *et al.*, 2019). The acidity of the berries declined significantly with ripening, and the decrease was higher in the warmer year 2020 compared with the colder year 2019 for all three studied varieties. The reduction of the TA in grapes during maturation is related to the respiration of the grape (Ferrero-del-teso *et al.*, 2020); especially in cold climate, the acid reduction is due to the use of malic acid as a substrate for respiration (Wati, 2019). Because this process is a function of the temperature (Wati, 2019), higher acid reductions occur in warmer years, as observed in our study. The TA of white grapes varieties generally ranges between 4 to 9 g/L tartaric ac. eq (Barnhill *et al*., 2018), but interspecific varieties grown in cold climate are known to have a higher TA when compared with *V. vinifera.* Thus, deacidification is a common practice in cold climate wine production (Slegers *et al*., 2015; Pedneault et al., 2013; Kemp *et al.*, 2018). On the other hand, the TSS content of berries increased significantly in all the three studied varieties. These results suggest that under Eastern Canada conditions, year to year specific climate variations, with higher accumulation of GDD in the year 2020 compared to 2019, positively impacted berries.

2.6.2 Grape volatile composition

Grape varietal aroma plays a crucial role in wine flavour. Varietal aroma compounds exist as free molecules in berries, but a larger proportion is found as non-volatile, odourless, bound forms that can be released by chemical and enzymatic reactions occurring during the wine making and wine ageing processes (Wu, *et al.*, 2014). These volatile compounds belong to many families, including monoterpenes, FADP and C_{13}-norisoprenoids (Hjelmeland and Ebeler, 2015).

The grape varieties analyzed in this study showed differences in berries' free and glycosylated aroma compounds. The FADP, which are responsible for herbaceous flavours, accounted for the highest percentage (approximately 93%) from the free-volatile fractions in all three

varieties studied. Similarly, Slegers *et al*. (2015) and Vilanova *et al*. (2012a) found that FADP accounted for the highest percentage from the free volatile compounds in interspecific hybrid grape varieties Frontenac, Marquette and Marechal Foch (about 93% in all three varieties) and in *Vitis vinifera* varieties Agudelo (90%) and Godello (99%) respectively.

Our study showed that 2-hexenal represented the largest portion of the total quantified FADP in L'Acadie blanc berries followed by 1-hexanal and (*E*)-2-hexenol. Vilanova *et al*., (2012a) also found (*E*)-2-hexenal and (*E*)-2-hexenol to account for the highest proportion of FADP in *Vitis vinifera* varieties Agudelo, Blanco lexitimo and Godello. The concentration of FADP of L'Acadie blanc especially, 2-hexenal and (*E*)-2-hexenol decreased significantly with ripening. García *et al.* (2003) observed a similar trend in *Vitis vinifera* varieties Airen, Macabeo and Chardonnay. Although the concentration of (*Z*) 3-hexenol did not show any distinct pattern in our results, García *et al.*, (2003) observed a decrease in the concentration of (*E*)-2-hexenol in the must and in (*Z*)-3-hexenol in berries of the white *Vitis vinifera* Airen, Macabeo and Chardonnay. In contrast, Kalua & Boss, (2010) showed that (*E*)-2-hexenal concentration in Riesling berries significantly increased after véraison until harvest. Since C_6-compounds are related to variety genotype and can be formed through lipoxygenase activity from linoleic and linolenic acids present in grapes via C_6-aldehydes (Slegers *et al*., 2015), concentration may vary from one variety to another (Vilanova *et al.*, 2012). (*E*)-2-hexenol and (*Z*) -3-hexenol come from the reduction of their respective aldehydes by alcohol dehydrogenase. The reduction in their concentrations with ripening could relate to a decrease in alcohol dehydrogenase activity (García *et al.*, 2003) or to the conversion of the alcohol into esters with the aid of alcohol acyltransferase enzymes (Wu *et al.*, 2020).

Free monoterpenes were only detected in Osceola Muscat and Seyval blanc berries. The highest level found was 324 µg/L in Osceola Muscat berries in the year 2020 at HD2 (1083 GDD) stage. On the other hand, bound monoterpenes were detected in all three studies varieties and the concentration significantly increased with ripening with a maximum value of 3520 µg/L at HD3, in Osceola Muscat, where the main type of monoterpene was (*Z*)-8-hydroxylinalool that accounted for 2690 µg/L at the same harvest stage. Similarly, the findings by Torchio *et al*. (2016) showed that the total concentration of bound terpenes increased significantly during ripening, showing the maximum value at a later stage at 20.2

°Brix for Moscato bianco grapes (V*itis vinifera L*). Although the bound monoterpene content was maximum on the last harvest, free monoterpenes were significantly higher at the second harvest when compared to the last harvest in Osceola Muscat in 2020, which could be related to fluctuations in temperature and water status caused due to low precipitation in the vineyard at the second harvest. It has been accepted that free monoterpenes could be easily affected by the alteration of climates during berry development stages due to their volatile property whereas, bound monoterpene composition is positively related to the level of total soluble solids in grapes (Li *et al.*, 2017). Osceola Muscat analyzed in our study showed the highest bound MTs contents due to the accumulation of MTs such as (*Z*)-linalool oxide (furanoid) and 1,6-octadien-3-ol, 3,7-dimethyl in higher quantities, which agree with previous results about the characteristic varietal aroma of cultivars belonging to the Muscat family (Zalacain *et al.*, 2007;Torchio *et al.*, 2016). The sum of monoterpenes was higher in 2020 than in 2019 in Osceola Muscat berries, suggesting that warmer conditions were more favorable for the synthesis of monoterpenes (Gaiotti *et al.*, 2014).

C_{13}-norisoprenoids were detected only in the bound fraction of volatile compounds. The concentration of C_{13} significantly increased with ripening in L'Acadie blanc berries harvested in 2019, whereas no trend was observed in other varieties and years. This result agrees with Ubeda *et al*. (2017) on *Vitis vinifera* cv País, showing that C_{13}-norisoprenoids increase with ripening. The C_{13}-norisoprenoids are thought to be formed as biodegradation products of carotenoids (Wu, 2019), and their concentrations increase during berry development as carotenoids degrade (Onofrio & Tomasi, 2013).

Bound volatile phenols and benzene derivatives composed the highest percentage of total bound volatile composition (51%) in L'Acadie blanc and benzyl alcohol and 2-phenylethanol were the major compounds. The concentration of these compounds decreased with ripening in L'Acadie blanc harvested in 2019. On the other hand, 2-phenylethanol was detected as the major volatile phenols and benzene derivative in the free volatile fraction of Osceola Muscat and Seyval blanc berries but was not detected in L'Acadie blanc berries. Similar results were found by Vilanova *et al.*, 2012, and Fenoll *et al.*, 2009 on *B. Lexitimo* and Muscat Hamburg grapes, where these compounds also decreased during ripening. Results of García *et al.*, (2003) suggest that the decrease of these compounds during the ripening of Macabeo, Airén

and Chardonnay musts could be attributed to the dilution effect produced by water accumulation in the grape during this period.

Furaneol was detected only in bound volatile fraction of Osceola Muscat berries and the concentration increased significantly with ripening. Compared to the colder year 2019, the warmer year 2020 resulted in a higher concentration of furaneol in the bound fraction of volatile compounds. Furaneol is formed via Maillard reaction, and thus is positively correlated with the concentration of sugar and higher temperature (Zhu *et al.*, 2019). Thus, furaneol content increased with berry ripening due to the increase in the sugar concentration and at the same time, due to the higher accumulation of GDD in 2020, accumulation was higher compared to 2019.

2.6.3 Wine volatile composition

The aroma is one of the main factors related to the quality of white wines and certain compounds involved in wine aroma should be taken into account to evaluate the optimal stage of grape ripening (Vilanova *et al.*, 2012). In the current study, fermentation-related volatile compounds such as fatty acid ethyl esters, free fatty acids and other fermentation products accounted for 98% of total volatile compounds quantified in the wines of the analyzed varieties. In contrast, berry related volatile compounds such as fatty acid degradation products (FADP) accounted for approximately 0.3%, and monoterpenes represented very little percentage of less than 0.05% of total volatiles. It is well known that a large proportion of wine volatiles is produced during fermentation and are related to yeast strain (Moreno Luna *et al.*, 2018). However, the proportion of volatiles compounds contributed by berries is highly characteristic of the grape variety (Slegers *et al.*, 2015).

Terpenes and norisoprenoids are derived from grapes and contribute to the "varietal aroma" of wines (Gao *et al.*, 2019). Only one terpene compound was detected in the studied wine samples (*β*-linalool), and this was only detected in wines from Osceola Muscat berries in warmer year 2020. The concentration of *β*-linalool increased significantly with ripening in Osceola Muscat wines from warmer year 2020. This agrees with previous authors stating that biosynthesis of monoterpenes increase at high temperatures (González-Barreiro *et al.*, 2015) and increase with ripening (Zhao *et al*., 2019). None of the C_{13}-norisoprenoids compounds quantified in berries were detected in analyzed wines. Zhao *et al*. (2019) detected three

terpenes and one norisoprenoid in Cabernet Sauvignon wines, including citronellol, linalool, geraniol, and *β*-damascenone, with relatively low thresholds and, similar to our findings, the linalool concentrations tended to increase with sequential harvesting. Similarly, the linalool concentration of wines from Pinot gris grapes increased with maturity but, in this case, the increase was likely a result of berry dehydration (Moreno Luna *et al.*, 2018). Although monoterpenes were detected in higher concentrations in the bound fractions of the analyzed berries, the low content of monoterpenes in wines is likely attributable to the winemaking process. In our study, skin maceration was not performed and, soon after destemming, berries were directly taken for pressing. Thus, the juice had limited contact with berry skin and a little extraction of monoterpenes occurred. Since a large proportion of the total monoterpenes are found in the berry skin (Park *et al*., 1991) extended skin contact before the pressing is an effective way to enhance monoterpene extraction, which results more floral notes in wine (Bindon *et al.*, 2013).

The concentration of fatty acid degradation products (FADP) such as (*Z*)-3-hexenol and 1-hexanol significantly decreased with ripening in studied berry varieties. Previous research by Gao *et al*. (2019) and Moreno Luna *et al*. (2018) found that harvesting berries later resulted in lower concentrations of C_6 alcohols. The C_6 alcohols are derived from C_{18} fatty acids via the lipoxygenase pathway and alcohol dehydrogenase, either *in situ* during grape ripening, or under the oxidative conditions present when the fruit is crushed (Bindon *et al.*, 2013). The reduction of their concentration in wines made from later harvest dates could relate to lower alcohol-dehydrogenase activity (García *et al.*, 2003) or conversion of C_6 alcohols into esters during wine fermentation (Wu *et al.*, 2020; Gao *et al.*, 2019).

Methyl butanol was the main bound aroma compound found in wine from all analyzed cultivars with an approximate percentage of 70% of total volatiles. The total concentration was higher in the warmer year 2020 when compared with the colder year 2019 (Supplemental materials, Table 2.10). The concentration of 3-methylbutanol significantly increased with ripening in Osceola Muscat berries in 2020. Similarly, the concentration of 3-methylpentanol significantly increased with ripening for all three varieties analyzed in this study. A similar trend was observed previously in wines made from *V. vinifera* variety Cabernet Sauvignon (Zhao *et al*., 2019) but, in contrast, Gao *et al*. (2019) found that as 3-methylbutanol and 3-

methylpentanol follow no consistent trend with ripening in the same grape variety. Other fermentation volatile compounds, specifically alcohols, are released into wine as secondary products of yeast metabolism and are synthesized via the two mechanisms of an anabolic pathway from glucose and a catabolic pathway from corresponding amino acids (Chang, Jung and Hur, 2014). An enhanced soluble solids concentration resulting from ripening provides more substrates and favorable environment for yeast metabolism and thus (Bindon *et al.*, 2013) could be associated with increased concentrations of these fermentation-related alcohols in wines (Moreno *et al*., 2018). Generally, if the concentration of these alcohols are less than 400 mg/L, it results in a positive wine aroma (Zhao *et al.*, 2019). Other than the alcohols, the concentration of isophenyl acetate increased significantly with ripening, in Osceola Muscat berries harvested in 2020. Similar to grape volatile compound, furaneol was only identified in Osceola Muscat wines from year 2020, and its concentrations significantly increased with ripening. Furaneol is a Maillard reaction product occurring when berries are exposed to heat (Slegers et al., 2015), which could explain its presence in Osceolat Muscat wines in 2020, which was much warmer than 2019.

Esters are important contributors to the wine aroma as they serve as a primary source of fruity aroma (Gayon *et al.*, 2006). Most esters are secondary metabolites from yeast metabolism (Wati, 2019). Out of the two categories of esters identified in wine (acetates of ethanol and higher alcohols, and esters of fatty acid metabolites and ethanol) (Moreno Luna *et al.*, 2018), acetate esters such as phenyl ethyl acetate and isoamyl acetate showed a significant variation with berry maturation and heat accumulation yearly. Indeed, according to our results, phenolic esters such as phenyl ethyl acetates were detected at very low levels in wine, but their concentrations significantly increased by 126% to 166% depending on the variety as berries ripened. The concentration of phenylethyl acetate and isoamyl acetate has been found to increase with ripening in Moristel wines (Ferrero-del-teso *et al.*, 2020). Such increases may be explicitly linked to the increase of TSS in subsequent fermentation and production of ethanol and higher alcohols, which elicit the synthesis of acetates from higher alcohols such as phenyl ethyl acetates (Moreno Luna *et al.*, 2018; Bindon *et al.*, 2013).

Major three fatty acids such as heptanoic, hexanoic and octanoic acids were detected in high concentrations in all three studies wines. These compounds did not show any distinct pattern

with ripening. But the results by Gao *et al.* (2019) on wines from Cabernet Sauvignon had observed that hexanoic and octanoic acid levels increased significantly with ripening. The concentration of total fatty acids in our experimental wines was around 1 to 8 mg/L. These fatty acids are related to negative flavors, an unpleasant fatty odor and even a rancid smell in wine when present at higher concentrations (>20 mg/L) however, they provide the smell of cheese and dairy-related flavour at concentrations of 4 to 10 mg/L (Zhao *et al.,* 2019; Moreno Luna *et al.*, 2018).

Overall, delaying harvest and higher GDD accumulation positively impacted most wine aroma compounds. This also relate to the accumulation of higher concentrations of sugars and lower concentrations of acids in berries which, on the other hand, affected the accumulation of volatile compounds in wines. Grape-related aroma compounds such as monoterpenes reached a maximum at the latest harvest stage in the warmer year, while the accumulation of FADPs was decreased. Furthermore, berries harvested later then sooner provided favorable conditions for yeast metabolism, resulting in desirable yeast-related aroma profile in wine.

2.6.4 Relating grape to wine

Understanding the relationships between berry volatile composition and how it is related with wine aroma composition is one of the ultimate goals of wine science. To improve our understanding of this relationship in hybrid varieties, we conducted redundancy analyses (RDA) using volatile compounds grouped by classes to relate the bound and free volatile compounds from berries to the volatile compounds of the resulting wines (Figure 2.5). Furthermore, the relation between grape and wine specific varietal aroma compounds, a second RDA was performed (figure 2.6).

The first RDA (Figure 2.5) showed a proper separation of grape varieties according to the grape bound, free and wine volatile compounds grouped by classes. There was a strong correlation between the sum of FADP in grape bound fraction and the sum of FADP in wine, but a negative correlation was found between the sum of FADP in grape-free fraction to the sum of FADP in wine. In detail, as shown in figure 2.6, hexanal, 1-hexanol, (*E*) 2-hexenol, (*E*) 2-hexenal, 2-heptanol and 3-hexenol accumulated in grape bound fraction were correlated with accumulation of 1-hexanol in wine. And a negative correlation was observed

between 1-hexanol in wine with (*E*)3-hexenol and (*Z*)3-hexenal of the free volatile fraction of berries. On the other side, a positive correlation was observed for the (*E*)3-hexenol and (*Z*)3-hexenal from free fraction of berries and (*Z*)3-hexenol in wine. FADP are generally accumulated during pre-fermentation steps of wine production, especially during crushing and maceration, due to activation of alcohol dehydrogenase enzyme (Robinson *et al.*, 2014). Yet the release of glycosylated precursors during fermentation also impacts the level of wine FADP (Ruiz *et al.*, 2019). The C_6 aldehydes such as (*E*)-2-hexenal and hexanal found in berry bound volatile components are formed at high concentrations in must by enzymatic oxidation of linolenic acid and are further reduced to their corresponding alcohols during winemaking (Waterhouse, Sacks and Jeffery, 2016). This could be the reason for the positive correlation of these aldehydes with respective alcohols in studied wine. Interestingly, FADP from grape bound fraction were correlated with accumulation of FADP in wine, which was characteristic of L'Acadie blanc, suggesting that the roles of these compounds in the final perception of wine aroma depend on the concentrations of these compounds depending on the grape variety (Oliveira *et al.*, 2008).

Compared to other studied interspecific varieties, Osceola Muscat wines showed significantly higher levels of monoterpenes, including *β*-linalool. And as shown in figure 2.5 RDA plot (B) sum of monoterpenes in grape bound and free fractions were strongly correlated with the accumulation of monoterpenes in wine, which was characteristic of Osceola Muscat. Since terpenes are responsible for the characteristic varietal aroma in white cultivars belonging to the Muscat family (Torchio *et al.*, 2016), this could be the reason for higher terpene accumulation in this variety. Accumulation of linalool in grape free and bound fraction was correlated with the accumulation of linalool in wine (Figure 2.6). Monoterpenes, can be found in both free and bound volatile fractions; however, higher fraction is found as non-volatile precursors linked to a sugar moieties (bound volatiles) than as free compounds in grapes and musts (Moreno Luna *et al.*, 2018). Hydrolysis of the glycoside precursor leads to the release of the free volatile aroma compound (Styger, Prior and Florian F. Bauer, 2011). The conversion of these compounds to free monoterpenoids can be carried out via acidic or enzymatic hydrolysis by enzymes (especially *β*-glucosidases) from grapes and/or microorganisms (non-*Saccharomyces* yeasts, *Saccharomyces* yeasts, and lactic acid bacteria) during the alcoholic and malolactic fermentation processes (Maicas and Mateo, 2005).

Although a higher concentration of monoterpenes such as (*Z*)-linalool oxide, linalool, nerol, 1,6-octadien-3-ol, 3,7-dimethyl, *L*-alpha-terpineol and geraniol were detected in the berry volatile fraction (Figure 2.6), only a single monoterpene compound (*β*-linalool) was detected in wine.

This can be explained with different reasons. One of the reasons would be due to the higher acidic condition in studied interspecific hybrid wines (maximum 13.4 g/L tartaric ac.eq.) and lower pH (2.5), the ability of *S. cerevisiae* to exhibits *β*-glucosidase activity may have declined. It has been shown that grape *β*-glucosidase enzymes exhibit optimal activity at pH 5 and with lower pH grape *β*-glucosidase is regarded as having a low contribution to the release of monoterpenoids from aglycones (Ruiz *et al.*, 2019). Other than that, yeast strain itself can impact the aroma profile of wine (Carrau *et al.*, 2005). We have used a single yeast strain *Saccharomyces cerevisiae* (Selectys L'eclatante), but using mixed strains has been shown to produce more monoterpenes and other volatile compounds in wine (Ruiz *et al.*, 2019) (Styger *et al.*, 2011). Another reason could relate with the winemaking protocol we used. After destemming, juices were immediately transferred for pressing without allowing skin maceration. It has been found that grape skins have a higher concentration of free and glycosylated monoterpenes than the flesh or juice (González-Barreiro *et al.*, 2015) and skin maceration had enhanced the monoterpene content in wines (Reynolds, Wardle and Dever, 2019).

Several studies have indicated that both the total available nitrogen and the balance of amino acids and ammonia can significantly affect the production of different groups of fermentation-derived volatile compounds (Ugliano *et al.*, 2007). Higher alcohols, which are directly related to amino acid metabolism in the cell is one of the main secondary volatile which is affected by YAN in berries (Ugliano *et al.*, 2007). In our study we noticed a significant increment in the concentration of higher alcohols as 3-methylbutanol and 2-phenylethanol in Osceola muscat berries. This could be explained by the increase in YAN and PAN content in Osceola Muscat berries with maturity. But, as we adjusted the nitrogen content of juice prior to fermentation for all the varieties, it is difficult to extrapolate definitive conclusions concerning the effect of nitrogen on wine aroma (Bindon *et al.*, 2013).

Overall, varietal aroma in grapes showed a correlation with varietal aroma in wine with distinct differences between varieties. Monoterpenes were highly accumulated in Osceola Muscat berries and the concentration increased with ripening. And at the same time, FADPs were highly accumulated in L'Acadie blanc berries, while the concentration decreased with ripening. Other fermentation derived volatile compounds were highly dependent on the initial nutrition content of berries (YAN, PAN) and the chemical properties of the wine matrix such as acidity, sugar content and pH. In white varieties, changes in the concentrations of volatile compounds during ripening differ with variety, making it more difficult to determine maturity based on varietal volatile content.

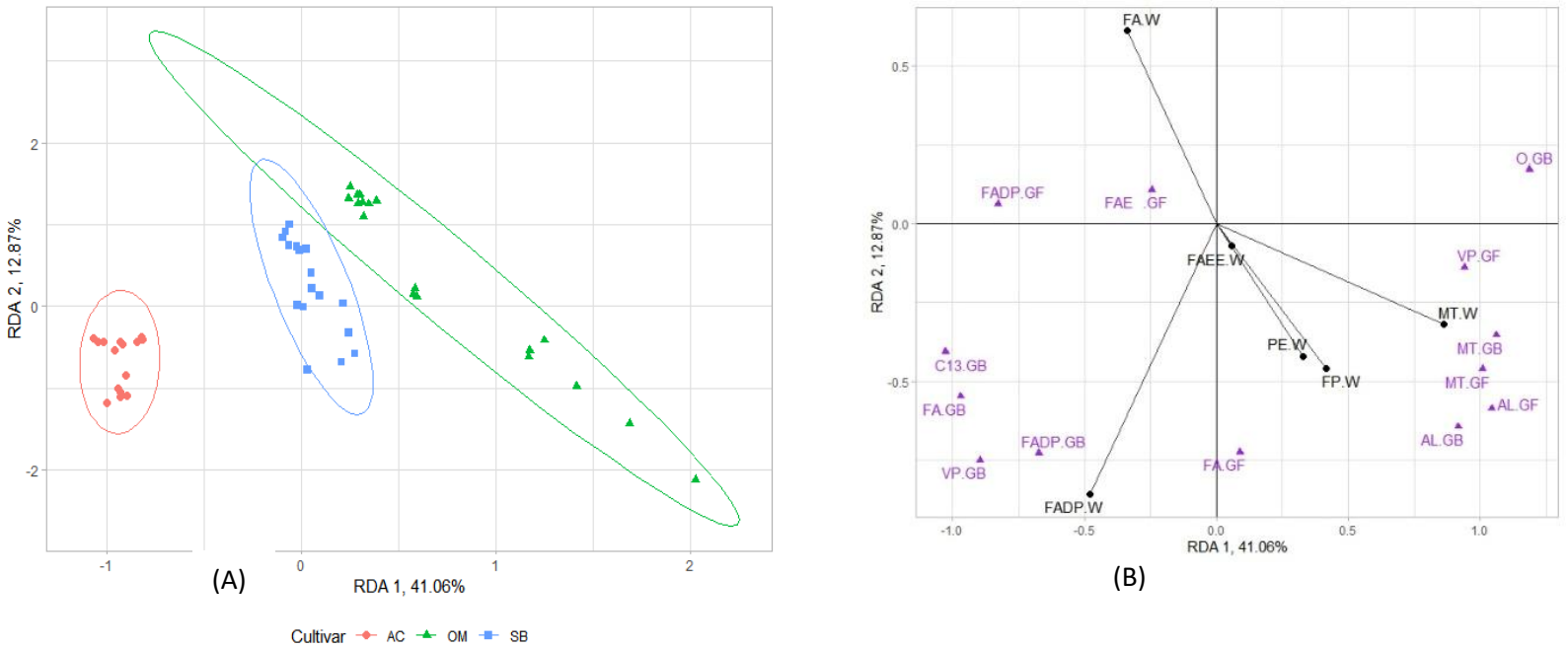


Figure 2.5 Redundancy analysis relating grape-free and bound volatile composition (independent variables) to volatile composition of wines (dependent variables) made from the interspecific hybrid grape varieties L'Acadie blanc (red diamonds), Osceola Muscat (green triangles) and Seyval blanc (blue squares). (A) Varieties samples plot (n =54); (B) Biplot of grape (purple triangles, 13 variables) and wine (blank circles, 6 variables). The berry free and bound volatile fractions are identified as the sum of monoterpenes in bound and free (MT.GB) (MT.GF), sum of volatile phenols in bound and free (VP.GB) (VP.GF), sum of alcohols in bound and free (AL GB) (AL GF), sum of fatty acid degradation products in bound and free (FADP.GB) (FADP.GF, sum of C13 norisoprenoids in bound (C13.GB), sum of fatty acids in bound and free (FFA.GB) (FFA.GF), sum of fatty acid esters in free (FAE.GF) and sum of other volatile compounds in bound (O.GB). The wine variables are identified as follows; sum of monoterpenes (MT. W), sum of fermented products (FP.W), sum of phenolic esters (PE.W), sum of fatty acid ethyl esters (FAEE.W), sum of fatty acids (FA.W) and the sum of fatty acid degradation products (FADP.W).

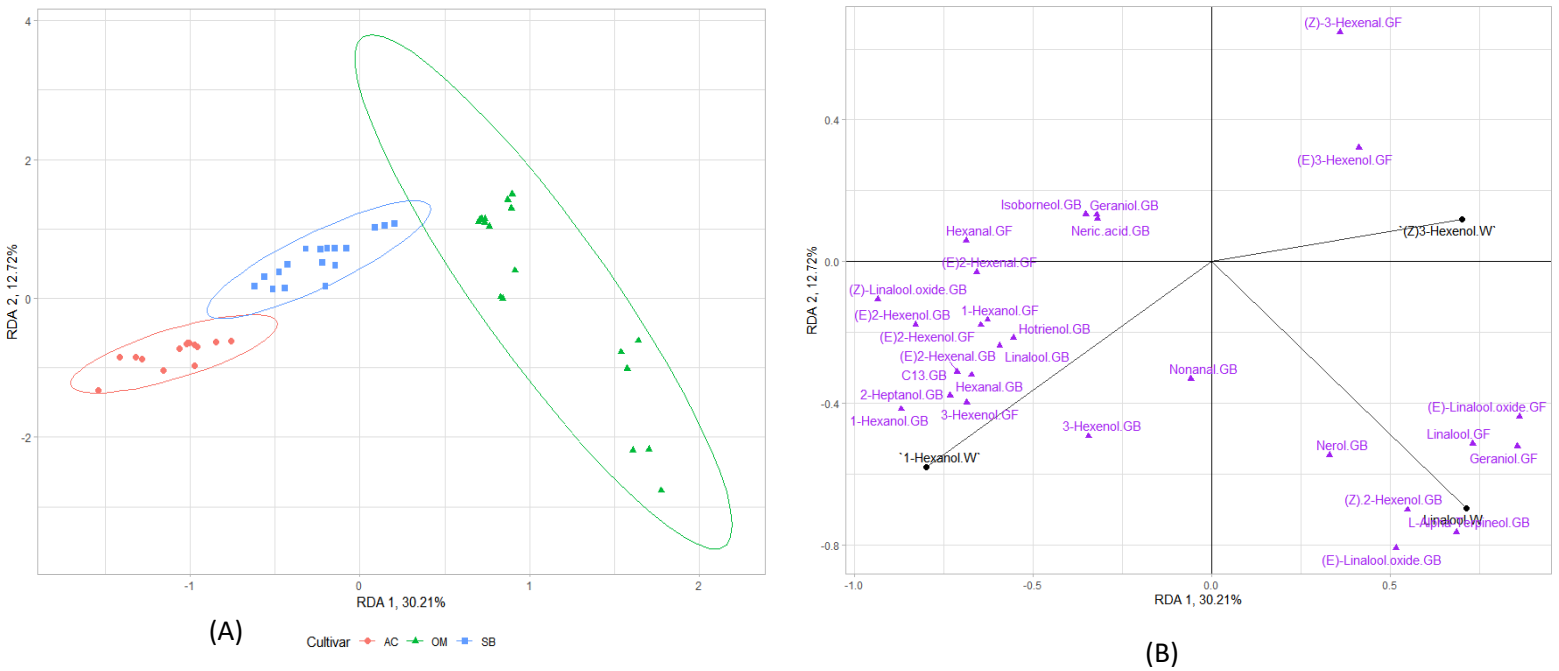


Figure 2.6 Redundancy analysis relating grape-free and bound volatile composition (independent variables) to volatile composition of wines (dependant variables) made from the interspecific hybrid grape varieties L'Acadie blanc (red diamonds), Osceola Muscat (green triangles) and Seyval blanc (blue squares). (A) Varieties samples plot (n =54); (B) Biplot of grape (purple triangles, 28 variables) and wine (blank circles, 3 variables). The berry bound volatile fractions (GB) are identified as C13 norisoprenoids in bound (C13.GB), Hexanal.GB, (E)2-Hexenal.GB, 3-Hexenol.GB, (E)2-Hexenol.GB, 1-Hexanol.GB, 2-Heptanol.GB, Nonanal.GB, (Z),2-Hexenol.GB, (Z)-Linalool oxide.GB, (E)-Linalool oxide.GB, Linalool.GB, Nerol.GB, L-Alpha-Terpineol.GB, Isoborneol.GB, Geraniol.GB, Neric acid.GB, Hotrienol.GB and grape free volatile fractions (GF) are identified as Hexanal.GF, (E)2-Hexenal.GF, 3-Hexenol.GF, (E)2-Hexenol.GF, 1-Hexanol.GF, (Z)-3-Hexenal.GF, (E)3-Hexenol.GF, Linalool.GF, (E)-Linalool oxide.GF and Geraniol.GF. Wine volatile compounds (W) are identified as Linalool.W, (Z)3-Hexenol.W and 1-Hexanol.W.

2.7 Conclusion

During this study, the profile of volatile compounds of berries and wines from interspecific hybrid *Vitis* sp. L'Acadie blanc, Osceola Muscat and Seyval blanc harvested at two vintages 2019 and 2020 from Nova Scotia were analyzed by SPE-GC-FID-MS. The results made it possible to distinguish the differences between three harvesting dates and varieties based on their berry and wine volatile compositions. Grape ripening stage significantly impacted the accumulation of wine volatile as aromatic esters (phenethyl acetate), monoterpenes (*β*-linalool), fatty acid ethyl esters, furaneol and *γ*-butyrolactone. On the contrary, the concentration of FADP such as *cis*-3-hexanol and 1-hexanol significantly decreased with ripening.

The results for berries showed significant differences between harvesting stages. The concentration of volatile compounds during berry development is regulated by metabolic production, expansion dilution, and conversion of free and bound volatile compounds (Wu *et al.*, 2020). Specific categories of volatile compounds such as FADP decreased with ripening while other compounds like terpenes and C_{13}-norisoprenoids increased. The sum of terpenes in both grape free and bound volatile compounds was strongly correlated with the presence of the terpene linalool in wine, which was characteristic of Osceola Muscat. Similarly, the sum of fatty acid degradation products (FADP) in grape-free and bound fractions were correlated with that of FADP in the wine and was characteristic for L'Acadie blanc. Among the three varieties analyzed in this study, Osceola Muscat showed valuable characteristics for cold-climate wine production in the warmer season at the latter harvesting stage with a significantly higher level of terpenes in wine.

For each variety, wine samples from the corresponding phenological stages had a lower acidity and higher total soluble solids in the warmer 2020 year when compared to those from 2019. In 2020, higher accumulation of GDD (warmer seasons) improved maturity (HD3) and positively impacted the volatile compound profile of wines produced from the studied interspecific hybrid *Vitis* varieties.

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2.9 Supplemental material

Table 2.9 Free volatile compounds (µg/L) from the berry of the interspecific hybrid grapes L'Acadie blanc, Osceola Muscat and Seyval blanc harvested in the Province of Nova Scotia (Canada) during the seasons 2019 and 2020. Each value represents the means of three harvesting dates (n=9). For a given variety and year, values followed by a different letter are significantly different at P≤0.05 according to Turkey's honestly significant difference test.

Compounds	**Acadie blanc**			**Osceola Muscat**			**Seyval blanc**		
Year	2019	2020	P value	2019	2020	P value	2019	2020	P value
Fatty acid degradation products (µg/L)									
(*E*)-2-Hexenal	3136 a	1212 b	≤0.0001	831.5	655.3	0.2401	1852	1595	0.3272
1-Hexanal	948.3 a	399.6 b	≤0.0001	245.67	172.4	0.1058	800.3 a	547.4 b	0.0019
(*E*)-2-Hexenol	225.8 a	108.9 b	0.0009	80.63	79.74	0.9533	142.8	147.9	0.8103
1-Hexanol	110.4 a	71.25 b	0.0034	58.49	56.23	0.8018	98.83	114.7	0.1232
(*Z*)-3-Hexenol	19.79 b	33.88 a	0.0022	0.6900 b	1.390 a	0.0433	nd	nd	-
(*E*,*E*)-2,4-Heptadienal	3.730	3.560	0.6029	1.060	1.500	0.1873	nd	nd	-
2-Ethyl-1-Hexanol	2.960	2.330	0.0736	nd	nd	-	2.900	3.010	0.6819
2-Octanone	2.510	2.530	0.8953	3.030	3.030	0.9887	2.520	2.610	0.3776
4-Decanol	2.580 b	4.430 a	0.0178	2.160	2.700	0.1561	4.140	4.590	0.5169
***E*,*E*-2,4-Hexadienal**	1.140	1.300	0.6436	nd	nd	-	1.040	0.8000	0.2907
(*Z*)-3-Hexenal	nd	nd	-	16.03	nd	-	nd	nd	-
(*E*)-3-Hexenol	nd	nd	-	61.46	53.25	0.2110	72.45	66.11	0.7706
1-Octene-3-ol	nd	nd	-	nd	nd	-	0.1200	0.4700	0.0338
Decanal	nd	nd	-	nd	nd	-	0.6600	1.310	0.0732

5-Ethyl 2-heptanol	nd	nd		1.79	3.43	0.0792	nd	nd	
SUM	4454 a	1841 b	≤0.0001	1303	1029	0.1919	2977	2484	0.1592
Alcohols (µg/L)									
(*Z*)-2-Pentenol	13.58	11.50	0.0610	nd	nd	-	nd	nd	-
(*E*)-2-Pentenol	nd	nd	-	2.630 b	4.770 a	≤0.0001	6.530 b	8.200 a	0.0359
2-Methyl 1-Butanol	nd	nd	-	2.570	8.660	0.0885	0.8500 b	13.18 a	0.0447
3-Methyl 1-Butanol	nd	nd	-	3.530	17.38	0.0636	1.190 b	17.55 a	0.0238
3-Methyl 3-Buten-1-ol	nd	nd	-	4.530	4.050	0.3878	nd	nd	-
SUM	13.58 a	11.50 b	0.0181	13.27 b	34.86 a	0.0181	8.570 b	38.93 a	0.0277
Fatty acids (µg/L)									
Octanoic acid	3.640	3.870	0.5004	1.270 b	5.800 a	≤0.0001	2.880	4.270	0.0554
Hexanoic acid	11.32	10.98	0.7989	4.730 b	9.650 a	≤0.0001	9.250	15.34	0.0522
SUM	14.97	14.78	0.0861	6.000 b	15.44 a	≤0.0001	12.13 b	19.61 a	0.0298
Fatty acid esters (µg/L)									
Octyl butyrate	6.430	5.110	0.1231	nd	nd	-	nd	nd	-
2-Pentyl propionate	nd	nd	-	nd	nd	-	14.71	15.43	0.8253
Sum	6.430 a	5.110 b	0.0140	nd	nd	-	14.71	15.43	0.8253
Volatile Phenols and benzene derivatives (µg/L)									
2-Phenyl ethanol	nd	nd	-	106.7 b	203.9 a	0.0020	37.34	92.20	0.1215
(mg/L)									
Benzeneacetaldehyde	12.27	17.61	0.1993	20.04 a	10.51 b	0.0482	13.04	11.24	0.4320

2-Phenoxy ethanol	7.070	7.710	0.5145	nd	nd	-	4.510 b	9.790 a	0.0007
Benzyl Alcohol	5.510 b	7.480 a	0.0411	28.54 a	23.82 b	0.0050	6.600 b	13.30 a	0.0361
Vanillin	4.390	4.130	0.6315	nd	nd	-	nd	nd	-
Benzophenone	2.820	2.860	0.7785	2.650 b	4.220 a	0.0012	2.330 b	3.460 a	0.0148
Benzaldehyde	1.040 b	1.870 a	≤0.0001	1.390 b	1.790 a	0.0384	0.920 b	1.640 a	0.0103
Vanillin, acetate	nd	nd	-	2.430	2.780	0.5398	nd	nd	-
3-Hydroxy-4-methoxybenzaldehyde	nd	nd	-	3.000 b	5.110 a	0.0005	4.560	5.540	0.4766
SUM	33.11	27.78	0.5099	164.7 b	252.2 a	0.002714	69.31	137.17	0.0957
Monoterpenes (µg/L)									
Linalool	nd	nd	-	3.220 b	53.22 a	0.0014	1.660	2.260	0.5140
(*E*)-Linalool oxide	nd	nd	-	1.170 b	5.090 a	0.0006	nd	nd	-
3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)	nd	nd	-	2.480 b	3.330 a	0.0015	nd	nd	-
Geraniol	nd	nd	-	1.900 b	6.640 a	0.0082	nd	nd	-
2,6-Dimethyl 2,7-Octadiene-1,6-diol	nd	nd	-	2.830 b	65.03 a	≤0.0001	6.160	7.640	0.3920
(*E*)-3,7-Dimethyl-, 2,6-	nd	nd	-	nd	nd	-	9.940	7.090	0.1605

Octadien-1-ol									
2,6-Dimethyl 3,7-Octadiene -2,6-diol	nd	nd	-	9.060 b	74.13 a	0.0002	nd	nd	-
2,6-Dimethyl 1,7-Octadiene -3,6-diol	nd	nd	-	nd	19.45	-	nd	nd	-
2,2-Dimethyl 4-Octen-3-ol	nd	nd	-	nd	nd	-	0.7900 b	1.490 a	0.0195
SUM	nd	nd	-	20.66 b	226.9 a	≤0.0001	18.55	18.48	0.9860

Table 2.10 Bound volatile compounds (µg/L) from the juice of the interspecific hybrid grapes L'Acadie blanc, Osceola Muscat and Seyval blanc harvested in the Province of Nova Scotia (Canada) during the seasons 2019 and 2020. Each value represents the means of three harvesting dates (n=9). For a given variety and year, values followed by a different letter are significantly different at P≤0.05 according to Turkey's honestly significant difference test.

	Variety								
Compounds	Acadie blanc			Osceola Muscat			Seyval blanc		
Year	2019	2020	P value	2019	2020	P value	2019	2020	P value
Fatty acid degradation products (µg/L)									
1-Hexanol	42.61	44.60	0.6156	8.220 b	16.27 a	0.0005	28.15 b	36.74 a	0.0130
(*E*)-2-Hexenal	34.45 b	89.05 a	≤0.0001	7.150b	20.33 a	0.0081	23.76 b	68.48 a	0.0013
***(Z)* 3-Hexenol**	19.47 b	29.51 a	≤0.0001	10.60 b	13.36 a	0.0401	5.050	5.170	0.8704
Nonanal	9.750	12.19	0.3368	7.110 b	13.34 a	≤0.0001	4.960 b	15.86 a	0.0148
(*E*)-2-Hexenol	6.800 b	11.61 a	0.0332	nd	nd	-	4.750 b	13.09 a	0.0004
Hexanal	6.330	7.990	0.2612	nd	nd	-	nd	nd	-
1-Pentanol	7.710	9.080	0.2163	nd	nd	-	nd	nd	-
2 Heptanol	3.410 b	4.830 a	0.0024	nd	nd	-	nd	nd	-
(*Z*)-2-Hexenol	nd	nd		nd	0.5500	-	nd	nd	-
SUM	130.5	208.9	0.8132	33.07 b	63.85 a	0.0003	66.67 b	139.3 a	0.0011
Alcohols (µg/L)									
2-Methyl 1-Butanol	29.66 b	35.04 a	0.0418	9.160	12.54	0.0723	29.17 b	45.91 a	0.0007
3-Methyl 3-Buten-1-ol	14.06 b	22.24 a	0.0002	14.33 b	23.14 a	0.0219	17.95 b	38.33 a	≤0.0001
3-Methyl 1-Butanol	14.12 b	19.43 a	≤0.0001	10.14 b	20.17 a	≤0.0001	22.07 b	36.83 a	≤0.0001
3-Methyl 2-Butenol	4.180 b	10.39 a	0.0407	1.790 b	5.330 a	≤0.0001	16.53 b	20.77 a	0.0341
Heptan-2-ol	3.970	5.390	0.0956	nd	nd	-	nd	nd	-

SUM	65.99	92.48	0.7883	35.43 b	61.18 a	0.0005	85.73 b	141.84 a	≤0.0001
Fatty acids (µg/L)									
Hexanoic acid	4.28 b	6.08 a	0.0408	nd	nd	-	nd	nd	-
Monoterpenes (µg/L)									
8-Hydroxylinalool	317.08 a	200.29 b	0.0108	nd	nd	-	nd	nd	-
***(Z)*-Linalool oxide**	34.33	27.62	0.1656	nd	nd	-	27.38 b	33.68 a	0.0019
(*E*)-Linalool oxide	24.32 a	12.05 b	0.0000	9.940 b	45.34 a	0.0082	15.60	14.70	0.3467
Hotrienol	17.03	18.35	0.8231	nd	nd	-	nd	nd	-
Linalool	9.330	9.700	0.8773	nd	nd	-	nd	nd	-
Nerol	9.970	6.210	0.0856	nd	25.20	-	19.73	17.79	0.7262
3,7-Dimethyl 1,6-Octadien-3-ol	nd	nd	-	7.010 b	59.60 a	0.0038	10.32	15.10	0.1858
***L*-Alpha-Terpineol**	nd	nd	-	nd	7.960	-	nd	nd	-
2,6-Dimethyl 2,7-Octadiene-1,6-diol	nd	nd	-	383.8 a	1424.4 b	0.0144	629.86	647.0	0.9114
Isoborneol	nd	nd	-	nd	nd	-	2.450 b	3.450 a	0.0107
Geraniol	nd	nd	-	nd	nd	-	90.48	124.97	0.1376
Neric acid	nd	nd	-	nd	nd	-	13.80	19.28	0.1891
2,6-Dimethyl 1,7-Octadien-3,6-diol	nd	nd	-	nd	12.86	-	nd	nd	-
2,6-Dimehtyl-1,7-octadiene-3-ol	nd	nd	-	36.89 b	153.8 a	0.0275	12.20	8.350	0.1825
Lilac alcohol C	4.450	4.040	0.7439	nd	nd	-	nd	nd	-
Lilac alcohol B	nd	nd	-	13.14 b	25.47 a	0.0038	nd	nd	-
3-Buten-2-ol, 4-2,6,6-trimethyl-2-cyclohexen-1-yl	17.51 b	40.23 a	0.0014	22.87 a	10.33 b	0.0090	nd	nd	-

2-Butanone, 4-(2,6,6-trimethyl-2-cyclohexen-1-yl)(R)	nd	nd	-	55.42 a	29.86 b	≤0.0001	27.70 b	36.27 a	0.0060
2-Cyclohexen-1-one, 4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl	nd	nd	-	162.4 a	128.3 b	0.0022	40.70 b	52.32 a	0.0044
2-Cyclohexen-1-one, 3-(3-hydroxybutyl)-2,4,4-trimethyl	nd	nd	-	8.410	8.070	0.6444	8.410 b	13.40 a	≤0.0001
2-Butanone, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)	nd	nd	-	nd	nd	-	129.1 b	151.74 a	0.02949
SUM	434.0 a	318.5 b	0.0056	699.9 b	1931 a	0.0189	1027	1138	0.5349
Volatile phenols and benzene derivatives (µg/L)									
Benzyl alcohol	639.9 b	786.0 a	≤0.0001	163.1 b	198.4 a	0.0056	427.3 b	528.7 a	0.0142
2-Phenylethanol	308.0 b	412.9 a	0.0057	161.6 b	246.5 a	0.0002	191.2 b	269.4 a	0.0092
Methyl vanillate	89.91 a	30.84 b	0.0020	36.80	72.65	0.1711	60.62	43.21	0.1290
***m*-Toluic acid, 3-tridecyl ester**	30.19	30.22	0.9904	nd	nd	-	nd	nd	-
Eugenol	13.06	15.18	0.1905	13.62 b	22.84 a	0.0099	nd	nd	-
p-Vinylguaiacol	12.50 a	5.130 b	0.0012	25.74	24.37	0.8719	14.94	8.560	0.1055
6-Methoxy eugenol	5.500	4.970	0.2927	nd	nd	-	nd	nd	-
3-Hydroxy-4-methoxy benzaldehyde	nd	nd	-	nd	1.400	-	nd	nd	-

Phenol, 2-methoxy-4-(1-propenyl)	nd	nd	-	22.67 a	17.57 b	0.0406	nd	nd	-
4-Hydroxy-3-methoxy benzenemethanol	nd	nd	-	nd	4.330	-	nd	nd	-
SUM	1099	1285	0.2946	423.5 b	588.02a	0.0020	694.0 b	849.8 a	0.0133
C13 Norisoprenoids (µg/L)									
3-Oxo-*α*-ionol	137.0	145.5	0.2268	nd	nd	-	nd	nd	-
***β* IONOL**	119.4 a	72.68 b	≤0.0001	nd	nd	-	nd	nd	-
3-Oxo-7,8-dihydro-*α*-ionol	93.29	99.29	0.0973	34.50 b	41.36 a	0.0080	nd	nd	-
3-Hydroxy-*β*-damascone	92.73	89.85	0.5713	60.60 a	50.80 b	0.0300	75.80	71.81	0.4358
3-Hydroxy-7,8-dihydro-*β*-ionol	37.30	34.16	0.3782	nd	nd	-	30.48	31.20	0.7553
3-Hydroxy-5,6-epoxy- *β* -ionone	15.43	13.82	0.2007	nd	nd	-	nd	nd	-
Dihydro-3-oxo-*β*-ionol	9.36	9.04	0.8375	nd	nd	-	nd	nd	-
SUM	504.5 a	464.3 b	0.0373	95.10	92.16	0.5417	106.3	103.0	0.6564
Other volatile compounds (µg/L)									
2-Butyltetrahydro furan,	6.020 a	4.660 b	0.0005	5.750	7.580	0.0791	nd	nd	-
Furaneol	nd	nd	-	4.770 b	97.01 a	0.0427	nd	nd	-
SUM	6.020 a	4.660 b	0.0060	10.51 b	104.6 a	0.0431	nd	nd	-

Table 2.11 Free volatile compounds of wines produced by interspecific hybrid grapes L'Acadie blanc, Osceola Muscat and Seyval blanc harvested in the Province of Nova Scotia (Canada) during the seasons 2019 and 2020. Each value represents the means of three harvesting dates (n=9). For a given variety and year, values followed by a different letter are significantly different at P≤0.05 according to Turkey's honestly significant difference test.

	Variety								
Compounds	Acadie blanc			Osceola Muscat			Seyval blanc		
Year	2019	2020	P value	2019	2020	P value	2019	2020	P value
Fatty acid degradation products (µg/L)									
(*Z*)-3-Hexenol	106.8	91.46	0.2598	341.4	515.0	0.1106	127.5 a	79.84 b	0.0274
1-Hexanol	1214 b	1540 a	0.0024	248.83b	620.3 a	≤0.0001	683.3 b	1115 a	0.0008
SUM	1321 b	1631 a	0.0075	590.2 b	1135 a	≤0.0001	720.7 b	1195 a	0.0033
Fatty acid ethyl esters (µg/L)									
Diethyl butanedioate	823.9 a	369.4 b	≤0.0001	962.2 a	499.3 b	≤0.0001	1164 a	373.2 b	≤0.0001
Ethyl octanoate	606.0	656.6	0.5192	288.4 b	739.7 a	0.0002	534.6	663.3	0.3865
Diethyle 2-hydroxypentanedioate	580.0 a	348.3 b	≤0.0001	448.8 a	242.9 b	≤0.0001	527.3 a	377.3 b	0.0003
Ethyl hexanoate	227.8	220.9	0.7653	155.0	162.6	0.6214	151.3 b	212.9 a	0.0032
Ethyl 2-oxopropanoate	180.5 a	99.69 b	0.0012	263.7	nd		178.3 a	114.6 b	0.0487
Ethyl butanoate	125.7	125.9	0.9820	79.20	92.88	0.1094	103.2	100.9	0.8656
Ethyl 2-hydroxy-3-phenylpropanoate	96.92	101.6	0.5080	86.24 b	121.7 a	0.0237	105.8	124.0	0.1361
Ethyl 4-hydroxybutanoate	77.88 b	276.2 a	≤0.0001	52.01 b	558.1 a	0.0048	74.60 b	401.1 a	0.0035
Ethyl 2-methylprpanoate	61.09	56.41	0.4151	57.65	67.65	0.2898	72.54	72.79	0.9760
Ethyl propanoate	68.54	85.10	0.4677	58.90 b	82.84 a	0.0025	67.87	66.55	0.8736
Ethyl 2-hydroxy-4-methylpentanoate	55.52	80.71	0.1597	62.63 b	156.9 a	0.0039	57.69	85.28	0.0980

Ethyl 3-methylbutanoate	38.67 b	53.11 a	≤0.0001	24.25 b	79.69 a	0.0005	39.77	45.79	0.2334
Ethyl decanoate	35.44	36.31	0.8578	20.08	22.35	0.6328	12.39 b	44.53 a	≤0.0001
Ethyl 3-hydroxybutanoate	44.08 b	62.54 a	0.0193	34.17 b	43.76 a	0.0059	41.48	53.06	0.0927
Ethyl 3-methyl butyl butanedioate	21.07 a	8.060 b	≤0.0001	26.42 a	9.28 b	≤0.0001	38.22 a	12.51 b	0.0023
Ethyl 3-hydroxypropionate	21.82 a	10.81 b	≤0.0001	19.67	38.90	0.0879	35.14 a	11.58 b	0.0019
Ethyl 2-methylbutanoate	7.760 a	4.99 b	≤0.0001	7.320	6.780	0.5243	9.140	7.490	0.2604
SUM	3072 a	2596 b	0.0032	2646	2925	0.1298	2856	2767	0.8253
Fatty acids (µg/L)									
2,4-Hexadienoic acid	4723	nd		4869	nd		4558	nd	
Octanoic acid	2301 a	609.5 b	≤0.0001	1475 a	513.2 b	≤0.0001	1620 a	565.0 b	0.0011
Hexanoic acid	949.7 a	440.5 b	≤0.0001	471.2 a	392.5 b	0.0389	697.7 a	416.3 b	0.0026
Isobutyric acid (mg/L)	334.8	317.1	0.5822	354.5	420.4	0.2042	461.4	422.1	0.5808
Heptanoic acid	112.8 a	37.54 b	≤0.0001	52.33	56.06	0.2439	104.6 a	43.85 b	≤0.0001
3-Methylbutanoic acid	139.0 b	206.8 a	0.0013	150.4 b	221.2 a	0.0168	161.7	229.9	0.0529
9 Decenoic acid	70.78 a	7.340 b	0.0006	66.44	60.11	0.5412	108.4 a	8.480 b	0.0012
2-Methylbutanoic acid	85.73	99.97	0.6477	66.70	119.2	0.1327	88.69	162.9	0.2380
Butanoic acid	nd	nd	-	nd	144.3	-	nd	nd	-
SUM	8718 a	1718.8 b	≤0.0001	7507 a	1927 b	≤0.0001	6934 a	1848 b	0.0034

Monoterpene (µg/L)									
***β*-Linalool**	nd	nd	-	nd	32.01	-	nd	nd	-
Phenolic esters (µg/L)									
Phenethyl acetate	27.85 b	132.6 a	0.0019	43.20 b	90.43 a	0.0037	40.27 b	133.4 a	0.0023
Other fermentation volatile compounds (µg/L)									
3-Methylbutanol	93857 b	131784 a	0.0031	80664 b	127437 a	0.0037	110779 b	159855 a	0.0033
2-Phenylethanol	23748 b	48592 a	0.0105	33097 b	51948 a	0.0196	30958 b	55601 a	0.0014
Diethyl malate	3759a	792.1 b	≤0.0001	4737 a	1333 b	≤0.0001	6497 a	1394b	≤0.0001
Ethyl lactate	2032 a	1550 b	0.0002	1690	1717	0.7982	2087 a	1578 b	0.0054
***γ*-Butyrolactone**	543.04a	391.4 b	≤0.0001	432.7	367.5	0.0662	553.9	474.4	0.0690
Tartaric acid diethyl ester	212.3 a	34.93 b	≤0.0001	355.8 a	81.99 b	≤0.0001	387.2 a	54.83 b	≤0.0001
2 Ethyl hexanol	345.5	202.3	0.2233	239.5	nd	-	305.2	164.1	0.3346
Isophenyl acetate	102.8 b	555.3 a	□□0.0001	92.20 b	307.1 a	0.0003	133.1 b	558.1 a	0.0012
***α* Butyrolactone**	104.6 b	138.4 a	0.0335	117.37 b	267.7 a	0.0106	105.7 b	158.5 a	0.0052
Diethyl succinate	91.10	102.6	0.1804	76.58 b	94.23 a	0.0366	94.33	85.69	0.5333
Pantolactone	62.19 b	82.85 a	0.0256	46.75	72.99	0.1121	76.28	108.9	0.1121
3-Methyl pentanol	72.52 b	142.2 a	0.0015	38.40 b	77.99 a	0.0018	59.23 b	116.8 a	0.0021
2-Tert-butyl-5-propyl-1,3-dioxolan-4-one	7.120 a	4.350 b	0.0054	4.890 b	87.33 a	≤0.0001	8.320	7.320	0.5140
3,4-Dimethyl 2-hexanol	63.60 a	18.10 b	≤0.0001	15.93	15.18	0.6083	63.84 a	14.84 b	0.0000
4-Methyl 1-pentanol	42.80	55.30	0.0883	21.15 b	43.72 a	0.0013	44.49 b	60.96 a	0.0123
2-Ethylbutanol	26.02 b	64.78 a	0.0011	15.07 b	82.57 a	≤0.0001	34.68 b	72.15 a	0.0005

2,6 Di (T-Butyl)-4-hydroxy-4-methyl-2,5 cyclohexadien-1-one	17.02	19.54	0.2877	25.73	24.55	0.6959	25.39	23.07	0.5469
Ethyl 2-hydroxyisovalerate	4.570	5.960	0.2185	4.530	6.290	0.0864	4.410	6.630	0.1115
Furaneol	nd	nd	-	nd	37.43	-	nd	nd	-
SUM	125092 b	184537 a	0.0062	121676 b	184004 a	0.0082	135305 b	220336 a	0.0038

Interaction between year and harvest date

2.12 Interactions between years and harvest dates for three interspecific hybrid grapes L'Acadie blanc, Osceola Muscat and Seyval blanc harvested in the Province of Nova Scotia (Canada)

Compounds	L'Acadie blanc	Osceola Muscat	Seyval blanc
Grape			
Berry weight (g)	0.0370	0.2392	0.0103
Cluster weight (g)	0.3958	<.0001	≤0.0001
TSS (° Brix)	<.0001	<.0001	<.0001
pH	<.0001	<.0001	<.0001
TA (g/L, tartaric ac eq.)	<.0001	<.0001	≤0.0001
PAN (mg/L)	<.0001	<.0001	<.0001
YAN (mg/L)	<.0001	<.0001	0.0001
Alcohol % v/v	0.2510	<.0001	0.0001
TA (g/L, tartaric ac. eq.)	<.0001	<.0001	<.0001
pH	<.0001	<.0001	<.0001
Free volatile compounds from grapes			
(*E*)-2-Hexenal	0.0001	<.0001	<.0001
1-Hexanal	<.0001	<.0001	0.0009
(*E*)-2-Hexenol	<.0001	<.0001	<.0001
1-Hexanol	0.0004	<.0001	0.0003
(*Z*)-3-Hexenol	0.0014	0.2496	0.0090
(*E,E*)-2,4-Heptadienal	0.1332	0.0034	<.0001
2-Ethyl-1-Hexanol	0.0024	0.0005	0.7269
2-Octanone	0.4539	0.5457	0.1271
4-Decanol	0.0398	0.0005	0.0073
E,E-2,4-Hexadienal	0.0003	<.0001	<.0001
(*Z*)-3-Hexenal	0.0002	<.0001	<.0001
(*E*)-3-Hexenol	0.0002	<.0001	0.0007
1-Octene-3-ol	0.0002	0.0037	<.0001
Decanal	0.0002	0.0037	0.0155
5-Ethyl 2-heptanol	0.0024	<.0001	0.7269
SUM	<.0001	<.0001	0.0001
(*Z*)-2-Pentenol	0.2393	<.0001	0.0001
(*E*)-2-Pentenol	0.2393	0.0010	<.0001
2-Methyl 1-Butanol	0.2393	0.0001	0.0001
3-Methyl 1-Butanol	0.2393	<.0001	<.0001
3-Methyl 3-Buten-1-ol	0.2393	0.7340	0.0001
SUM	0.0001	<.0001	0.0128
Octanoic acid	0.5738	0.0002	0.0002
Hexanoic acid	0.3773	0.0062	<.0001
Octyl butyrate	0.4416	0.0178	<.0001
2-Pentyl propionate	0.4416	0.0178	0.0043
SUM	0.0006	0.0178	0.0043
2-Phenyl ethanol	0.1349	0.0037	<.0001
Benzeneacetaldehyde	0.0045	<.0001	0.0009
2-Phenoxy ethanol	0.0778	0.0002	<.0001
Benzyl Alcohol	0.0211	0.0002	<.0001
Vanillin	0.0028	0.0178	0.0043

Benzophenone	0.1349	<.0001	0.0002
Benzaldehyde	0.0006	0.0022	0.0066
Vanillin, acetate	0.0778	0.0178	<.0001
3-Hydroxy-4-methoxy benzaldehyde	0.0028	0.0001	<.0001
SUM	<.0001	0.0001	<.0001
Linalool	0.0028	<.0001	0.0001
(*E*)-Linalool oxide	0.0028	<.0001	0.0001
3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)	0.0028	0.0006	0.0001
Geraniol	0.0028	<.0001	0.0001
2,6-Dimethyl 2,7-Octadiene-1,6-diol	0.0028	<.0001	<.0001
(*E*)-3,7-Dimethyl-, 2,6-Octadien-1-ol	0.0028	<.0001	0.0001
2,6-Dimethyl 3,7-Octadiene-2,6-diol	0.2393	<.0001	<.0001
2,6-Dimethyl 1,7-Octadiene-3,6-diol	0.0024	<.0001	0.7269
2,2-Dimethyl 4-Octen-3-ol	0.0024	<.0001	<.0001
SUM	<.0001	<.0001	<.0001
Bound volatile compounds from grapes			
1-Hexanol	0.0005	0.0003	<.0001
(*E*)-2-Hexenal	0.0002	<.0001	<.0001
(Z) 3-Hexenol	<.0001	0.0006	0.0582
Nonanal	0.0178	0.0038	0.0002
(*E*)-2-Hexenol	<.0001	0.0006	<.0001
Hexanal	0.0714	<.0001	0.0002
1-Pentanol	0.0005	0.0001	0.0003
2 Heptanol	0.0001	0.0003	<.0001
(*Z*)-2-Hexenol	0.0178	0.0038	0.0002
SUM	<.0001	0.0001	<.0001
2-Methyl 1-Butanol	0.0061	0.0001	0.0003
3-Methyl 3-Buten-1-ol	<.0001	0.0025	<.0001
3-Methyl 1-Butanol	<.0001	0.0002	<.0001
3-Methyl 2-Butenol	0.0003	<.0001	0.0002
Heptan-2-ol	0.0377	0.1015	0.0001
SUM	<.0001	0.0018	<.0001
Hexanoic acid	0.0009	0.0038	0.0002
8-Hydroxylinalool	<.0001	0.0038	0.0001
(Z)-Linalool oxide	0.0002	0.0038	0.0035
(*E*)-Linalool oxide	<.0001	0.0038	0.1962
Hotrienol	<.0001	<.0001	0.0002
Linalool	0.0002	0.0038	0.1962
Nerol	<.0001	0.0038	0.0001
3,7-Dimethyl 1,6-Octadien-3-ol	<.0001	<.0001	<.0001
L-Alpha-Terpineol	<.0001	<.0001	<.0001
2,6-Dimethyl 2,7-Octadiene-1,6-diol	<.0001	<.0001	0.0004
Isoborneol	<.0001	<.0001	<.0001
Geraniol	<.0001	<.0001	<.0001
Neric acid	<.0001	<.0001	<.0001

2,6-Dimethyl 1,7-Octadien-3,6-diol	<.0001	<.0001	<.0001
2,6-Dimehtyl-1,7-octadiene-3-ol	<.0001	<.0001	0.0002
Lilac alcohol C	<.0001	<.0001	<.0001
Lilac alcohol B	<.0001	<.0001	<.0001
3-Buten-2-ol, 4-2,6,6-trimethyl-2-cyclohexen-1-yl	0.0003	<.0001	<.0001
2-Butanone, 4-(2,6,6-trimethyl-2-cyclohexen-1-yl)(R)	0.0003	0.0001	<.0001
2-Cyclohexen-1-one, 4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl	0.0003	0.0008	0.0019
2-Cyclohexen-1-one, 3-(3-hydroxybutyl)-2,4,4-trimethyl	0.0003	0.0228	<.0001
2-Butanone, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)	0.0003	0.0228	0.0001
SUM	<.0001	<.0001	0.0004
Benzyl alcohol	0.0001	0.0132	<.0001
2-Phenylethanol	0.0001	0.0012	0.0168
Methyl vanillate	<.0001	<.0001	0.0305
m-Toluic acid, 3-tridecyl ester	<.0001	<.0001	0.0305
Eugenol	0.3297	0.0018	<.0001
p-Vinylguaiacol	0.0004	<.0001	<.0001
6-Methoxy eugenol	0.0311	0.0018	<.0001
3-Hydroxy-4-methoxy benzaldehyde	0.0001	<.0001	<.0001
Phenol, 2-methoxy-4-(1-propenyl)	0.0001	0.0923	<.0001
4-Hydroxy-3-methoxy benzenemethanol	0.0001	0.0923	<.0001
SUM	<.0001	0.0018	<.0001
3-Oxo-*α*-ionol	0.0009	0.0030	0.0007
β Ionol	<.0001	0.0030	0.0007
3-Oxo-7,8-dihydro-*α*-ionol	0.0858	<.0001	0.0007
3-Hydroxy-*β*-damascone	0.0030	0.0030	0.0002
3-Hydroxy-7,8-dihydro-*β*-ionol	0.0005	0.0030	0.0007
3-Hydroxy-5,6-epoxy- *β* -ionone	0.0012	0.0030	0.0007
Dihydro-3-oxo-*β*-ionol	<.0001	<.0001	0.0007
SUM	<.0001	0.1411	0.0003
2-Butyltetrahydro furan,	0.0001	<.0001	<.0001
Furaneol	<.0001	<.0001	0.0007
SUM	<.0001	<.0001	0.0003
Wine free volatile compounds			
(Z)-3-Hexenol	<.0001	<.0001	0.0003
1-Hexanol	<.0001	<.0001	0.0136
SUM	<.0001	<.0001	0.0340
Diethyl butanedioate	<.0001	<.0001	<.0001

Ethyl octanoate	0.8444	<.0001	0.5015
Diethyle 2-hydroxypentanedioate	<.0001	<.0001	0.0016
Ethyl hexanoate	0.0045	0.0024	0.0792
Ethyl 2-oxopropanoate	0.0002	<.0001	0.2813
Ethyl butanoate	0.7591	0.0212	0.1639
Ethyl 2-hydroxy-3-phenylpropanoate	0.0209	0.0069	0.1418
Ethyl 4-hydroxybutanoate	<.0001	<.0001	0.0031
Ethyl 2-methylprpanoate	0.0138	0.0003	0.3301
Ethyl propanoate	0.0853	0.0320	0.0004
Ethyl 2-hydroxy-4-methylpentanoate	0.0041	0.0330	0.3653
Ethyl 3-methylbutanoate	0.0001	0.0001	0.5248
Ethyl decanoate	0.0062	0.7949	0.0001
Ethyl 3-hydroxybutanoate	<.0001	0.0042	0.0008
Ethyl 3-methyl butyl butanedioate	<.0001	<.0001	0.0089
Ethyl 3-hydroxypropionate	<.0001	0.0078	0.0019
Ethyl 2-methylbutanoate	<.0001	0.0088	0.3439
SUM	0.0264	0.3653	0.5603
2,4-Hexadienoic acid	0.1723	0.0009	0.0458
Octanoic acid	0.0018	<.0001	0.0230
Hexanoic acid	<.0001	0.0273	0.1157
Isobutyric acid	0.0470	0.0073	0.0532
Heptanoic acid	0.0018	<.0001	0.0200
3-Methylbutanoic acid	0.0423	0.0008	0.0063
9 Decenoic acid	0.0002	0.0009	0.0005
2-Methylbutanoic acid	0.1723	0.0009	0.0458
Butanoic acid	0.0470	0.0073	0.0532
SUM	<.0001	<.0001	0.0248
β-Linalool	<.0001	<.0001	0.0364
Phenethyl acetate	<.0001	0.0001	0.0005
3-Methylbutanol	0.0003	0.0001	0.0259
2-Phenylethanol	<.0001	<.0001	0.0027
Diethyl malate	<.0001	<.0001	<.0001
Ethyl lactate	0.0001	0.0957	0.0075
γ-Butyrolactone	0.0001	0.0348	0.0239
Tartaric acid diethyl ester	<.0001	<.0001	<.0001
2 Ethyl hexanol	0.2454	<.0001	0.2464
Isophenyl acetate	<.0001	<.0001	0.0004
α-Butyrolactone	0.0121	0.0130	0.0584
Diethyl succinate	<.0001	<.0001	0.0364
Pantolactone	0.0264	0.0245	0.4109
3-Methyl pentanol	<.0001	<.0001	<.0001
2-Tert-butyl-5-propyl-1,3-dioxolan-4-one	0.0431	<.0001	0.3650
3,4-Dimethyl 2-hexanol	<.0001	0.9448	<.0001
4-Methyl 1-pentanol	<.0001	0.0001	0.0257
2-Ethylbutanol	0.0076	<.0001	0.0159
2,6 Di (T-Butyl)-4-hydroxy-4-methyl-2,5 cyclohexadien-1-one	0.0408	0.4121	0.6203

Ethyl 2-hydroxyisovalerate	0.1364	0.0001	0.3222
Furaneol	0.0408	0.4121	0.6203
SUM (mg/L)	<.0001	<.0001	0.0182

2.13 ANOVA table (P values) for basic physicochemical parameters of grape juice and wine from three interspecific hybrid grapes L'Acadie blanc, Osceola Muscat and Seyval blanc harvested in the Province of Nova Scotia (Canada) in 2019 and 2020.

Variety						
Compounds	Acadie blanc		Osceola Muscat		Seyval blanc	
P value						
Year	2019	2020	2019	2020	2019	2020
Alcohol % v/v	0.1700	0.5460	0.7700	0.0560	0.7720	0.0630
TA	0.0000	0.0000	0.0012	0.0000	0.0001	0.0032
(g/L, tartaric ac. eq.)						
pH	0.0001	0.0000	0.0004	0.0011	0.0113	0.0004
Berry weight (g)	0.0560	0.6950	0.3180	0.1580	0.0116	0.5090
Cluster weight (g)	0.6880	0.3780	0.0051	0.0018	0.0084	0.0589
TSS	0.0005	0.0000	0.0047	0.0038	0.0530	0.0002
(° Brix)						
pH	0.1490	0.0000	0.0066	0.0000	0.0777	0.0000
TA (g/L, tartaric ac eq.)	0.0000	0.0002	0.0003	0.0005	0.0558	0.0012
PAN (mg/L)	0.0004	0.0025	0.0008	0.0050	0.0018	0.0027
YAN (mg/L)	0.0004	0.0026	0.0012	0.0028	0.0010	0.2960

2.14 ANOVA table (P values) for free volatile compounds of grape juice from three interspecific hybrid grapes L'Acadie blanc, Osceola Muscat and Seyval blanc harvested in the Province of Nova Scotia (Canada) in two years 2019 and 2020.

Variety						
Compounds	Acadie blanc		Osceola Muscat		Seyval blanc	
P value						
Year	2019	2020	2019	2020	2019	2020
Fatty acid degradation products						
1-Hexanal	0.0004	0.5800	0.0000	0.0000	0.0900	0.0500
(*E*)-2-Hexenal	0.0000	0.9000	0.0000	0.0000	0.0000	0.0400
(*Z*)-3-Hexenol	0.0230	0.2500	0.4000	0.6600	nd	nd
(*E*)-2-Hexenol	0.0005	0.1670	0.0000	0.0000	0.0000	0.0900
1-Hexanol	0.0090	0.1300	0.0000	0.0000	0.0000	0.2200
E,E-2,4-Hexadienal	0.0540	0.0200	0.0100	nd	nd	0.0100
2-Octanone	0.4360	0.2300	0.5500	0.3100	0.1700	0.1700
(*E,E*)-2,4-Heptadienal	0.2130	0.1000	0.2700	0.0100	nd	nd
(*Z*)-3-Hexenal	nd	nd	0.0000	nd	nd	nd
(*E*)-3-Hexenol	nd	nd	0.0000	0.8000	0.0000	0.0900
1-Octene-3-ol	nd	nd	nd	nd	0.6300	0.0000
Decanal	nd	nd	nd	nd	0.0500	0.1400
4-Decanol	0.2410	0.3400	0.0100	0.0200	0.2600	0.0100
2-Ethyl-1-Hexanol	0.0504	0.0500	nd	nd	0.3400	0.9600
5-Ethyl 2-heptanol	nd	nd	0.0700	0.0000	nd	nd
SUM	0.0000	0.8100	0.0000	0.0000	0.0000	0.0500
Alcohols						
(*Z*)-2-Pentenol	0.4720	0.4800	nd	nd	nd	nd
3-Methyl 3-Buten-1-ol	nd	nd	0.6200	0.6200	nd	nd
3-Methyl 1-Butanol	nd	nd	0.0000	0.0000	0.0600	0.0000
2-Methyl 1-Butanol	nd	nd	0.0600	0.0000	0.0500	0.0100
(*E*)-2-Pentenol	nd	nd	0.2900	0.5700	0.0500	0.0000

SUM	0.4720	0.4800	0.0500	0.0000	0.3000	0.0000
Fatty acids						
Octanoic acid	0.6700	0.2600	0.0500	0.8900	0.0800	0.0000
Hexanoic acid	0.7800	0.1100	0.8400	0.1600	0.0000	0.2900
SUM	0.6000	0.1100	0.2800	0.4000	0.0000	0.1500
Fatty acid esters						
Octyl butyrate	0.5300	0.7800	nd	nd	nd	nd
2-Pentyl propionate	nd	nd	nd	nd	0.1400	0.0100
Sum	0.5300	0.7800	nd	nd	0.1400	0.0100
Volatile Phenols and benzene derivatives						
Benzaldehyde	0.3830	0.1500	0.0700	0.0600	0.2200	0.0700
Benzyl Alcohol	0.1430	0.0000	0.7500	0.0000	0.6900	0.0000
Benzeneacetaldehyde	0.0105	0.0400	0.0000	0.0000	0.0000	0.9400
Benzophenone	0.9470	0.0600	0.0000	0.1700	0.0100	0.0700
2-Phenyl ethanol	nd	nd	0.5300	0.1700	0.0700	0.0000
2-Phenoxy ethanol	0.0460	0.5400	nd	nd	0.0000	0.1700
Vanillin, acetate	nd	nd	0.0700	0.0800	nd	nd
Vanillin	0.0256	0.0100	nd	nd	nd	nd
3-Hydroxy-4-methoxy benzaldehyde	nd	nd	0.0800	0.0200	0.0000	0.1100
SUM	0.0800	0.0600	0.0700	0.2900	0.7700	0.0000
Monoterpenes						
Linalool	nd	nd	0.6000	0.0000	0.3000	0.1100
(*E*)-Linalool oxide	nd	nd	0.0500	0.0000	nd	nd
3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)	nd	nd	0.8000	0.0900	nd	nd
Geraniol	nd	nd	0.0100	0.0000	nd	nd
2,6-Dimethyl 2,7-Octadiene-1,6-diol	nd	nd	0.0000	0.0000	0.7000	0.0600
(*E*)-3,7-Dimethyl-, 2,6-Octadien-1-ol	nd	nd	nd	nd	0.0000	0.1300
2,6-Dimethyl 3,7-Octadiene-2,6-diol	nd	nd	0.6700	0.0000	nd	nd
2,6-Dimethyl 1,7-Octadiene-3,6-diol	nd	nd	nd	0.0100	nd	nd
2,2-Dimethyl 4-Octen-3-ol	nd	nd	nd	nd	0.8000	0.0100
SUM	nd	nd	0.1900	0.0000	0.0000	0.1100

2.15 ANOVA table (P values) for bound volatile compounds of grape juice from three interspecific hybrid grapes L'Acadie blanc, Osceola Muscat and Seyval blanc harvested in the Province of Nova Scotia (Canada) in two years 2019 and 2020.

Variety						
Compounds	Acadie blanc		Osceola Muscat		Seyval blanc	
P value						
Year	2019	2020	2019	2020	2019	2020
Fatty acid degradation products						
Hexanal	0.0570	0.4000	nd	nd	nd	nd
(*E*)-2-Hexenal	0.5000	0.4000	0.0550	0.0030	0.6000	0.0800
(Z) 3-Hexenol	0.0100	0.1000	0.0030	0.1300	0.6270	0.0800
(*E*)-2-Hexenol	0.0100	0.0039	nd	nd	0.0010	0.0000
1-Hexanol	0.0200	0.0000	0.2080	0.0920	0.0010	0.0070
2 Heptanol	0.0010	0.5900	nd	nd	nd	nd
Nonanal	0.0100	0.5300	0.1830	0.7330	0.0560	0.0130
(*Z*)-2-Hexenol	nd	nd	nd	nd	nd	nd
1-Pentanol	0.0020	0.0600	nd	nd	nd	nd
Sum	0.0510	0.3300	0.0600	0.0970	0.0600	0.0050
Alcohols						
3-Methyl 3-Buten-1-ol	0.0010	0.4700	0.0160	0.1140	0.0710	0.0540
3-Methyl 1-Butanol	0.4360	0.0600	0.0640	0.5640	0.3350	0.7930
2-Methyl 1-Butanol	0.0210	0.3300	0.0010	0.1380	0.0520	0.0810
3-Methyl 2-Butenol	0.0010	0.8700	0.0000	0.0560	0.0020	0.3080
Heptan-2-ol	0.1640	0.1100	nd	nd	nd	nd
SUM	0.3850	0.1200	0.0790	0.4300	0.0910	0.4370
Fatty acids						
Hexanoic acid	0.0040	0.1400	nd	nd	nd	nd
Monoterpenes						
(Z)-Linalool oxide	0.0060	0.0060	nd	nd	0.0270	0.4810
(*E*)-Linalool oxide	0.0570	0.9180	0.1100	0.0050	0.1280	0.4580
Linalool	0.0030	0.0050	nd	nd	nd	nd
Nerol	0.0000	0.0040	nd	0.1450	0.0010	0.0560
8-Hydroxylinalool	0.0000	0.0560	nd	nd	nd	nd

3,7-Dimethyl 1,6-Octadien-3-ol	nd	nd	0.2850	0.0040	0.0040	0.0000
L-Alpha-Terpineol	nd	nd	nd	0.0020	nd	nd
2,6-Dimethyl 2,7-Octadiene-1,6-diol	nd	nd	0.1350	0.0000	0.0040	0.0090
Isoborneol	nd	nd	nd	nd	0.0000	0.0560
Geraniol	nd	nd	nd	nd	0.0020	0.0020
Neric acid	nd	nd	nd	nd	0.0000	0.0670
Lilac alcohol C	0.0030	0.0000	nd	nd	nd	nd
Lilac alcohol B	nd	nd	0.3390	0.0020	nd	nd
Hotrienol	0.0000	0.0000	nd	nd	nd	nd
2,6-Dimethyl 1,7-Octadien-3,6-diol	nd	nd	nd	0.0800	nd	nd
2,6-Dimehtyl-1,7-octadiene-3-ol	nd	nd	0.0450	0.0000	0.0020	0.0630
3-Buten-2-ol, 4-2,6,6-trimethyl-2-cyclohexen-1-yl	0.0600	0.0940	0.4780	0.0000	nd	nd
2-Butanone, 4-(2,6,6-trimethyl-2-cyclohexen-1-yl)(R)	nd	nd	0.4100	0.1350	0.0000	0.5500
2-Cyclohexen-1-one, 4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl	nd	nd	0.0060	0.4290	0.0490	0.0900
2-Cyclohexen-1-one, 3-(3-hydroxybutyl)-2,4,4-trimethyl	nd	nd	0.9950	0.0130	0.0670	0.0780
2-Butanone, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)	nd	nd	nd	nd	0.0010	0.7850
SUM	0.0000	0.0060	0.1250	0.0000	0.0050	0.0160
Other volatile compounds						
2-Butyltetrahydro furan,	0.0020	0.4650	0.0030	0.0010	nd	nd
Furaneol	nd	nd	0.0040	0.0010	nd	nd
SUM	0.0020	0.4650	0.0030	0.0010	nd	nd
Volatile phenols and benzene derivatives						
Benzyl alcohol	0.0020	0.4920	0.0860	0.4220	0.0000	0.0000
2-Phenylethanol	0.0010	0.2950	0.5710	0.3230	0.6190	0.1270
Methyl vanillate	0.0000	0.7910	0.0000	0.0010	0.0400	0.1740

m-Toluic acid, 3-tridecyl ester	0.0000	0.1350	nd	nd	nd	nd
p-Vinylguaiacol	0.0080	0.7340	0.0020	0.0000	0.0000	0.1480
3-Hydroxy-4-methoxy benzaldehyde	nd	nd	nd	nd	nd	nd
Phenol, 2-methoxy-4-(1-propenyl)	nd	nd	0.4290	0.1670	nd	nd
4-Hydroxy-3-methoxy benzenemethanol	nd	nd	nd	nd	nd	nd
Eugenol	0.7160	0.2390	0.0690	0.0540	nd	nd
6-Methoxy eugenol	0.2600	0.4010	nd	nd	nd	nd
SUM	0.0030	0.6880	0.0020	0.1240	0.0020	0.0000
C13 Norisoprenoids						
3-Hydroxy-*β*-damascone	0.0040	0.4580	0.0060	0.9450	0.0010	0.2630
3-Hydroxy-7,8-dihydro-*β*-ionol	0.0010	0.2130	nd	nd	0.0020	0.0830
3-Oxo-*α*-ionol	0.0020	0.3130	nd	nd	nd	nd
β IONOL	0.0000	0.0670	nd	nd	nd	nd
3-Hydroxy-5,6-epoxy- *β* -ionone	0.0060	0.0560	nd	nd	nd	nd
3-Oxo-7,8-dihydro-*α*-ionol	0.0900	0.5920	0.0000	0.1310	nd	nd
Dihydro-3-oxo-*β*-ionol	0.0000	0.1090	nd	nd	nd	nd
SUM	0.0020	0.6970	0.0580	0.7900	0.0010	0.1530

2.16 ANOVA table (P values) for free volatile compounds of wines from three interspecific hybrid grapes L'Acadie blanc, Osceola Muscat and Seyval blanc harvested in the Province of Nova Scotia (Canada) in two years 2019 and 2020.

Variety						
Compounds	Acadie blanc		Osceola Muscat		Seyval blanc	
P value						
Year	2019	2020	2019	2020	2019	2020
Fatty acid degradation products						
(*Z*)-3-Hexenol	0.0000	0.0200	0.0000	0.0000	0.0100	0.0600
1-Hexanol	0.0000	0.0500	0.0800	0.0200	0.2500	0.5900
SUM	0.0000	0.0600	0.0000	0.0500	0.2500	0.5000
Fatty acid ethyl esters						
Ethyl propanoate	0.0840	0.2000	0.1300	0.9800	0.0000	0.9400
Ethyl 2-methylprpanoate	0.0800	0.0700	0.0600	0.0000	0.3200	0.2400
Ethyl butanoate	0.8260	0.3900	0.0700	0.0900	0.3900	0.0900
Ethyl 2-oxopropanoate	0.1770	0.0100	0.1200	nd	0.4600	0.6800
Ethyl 2-methylbutanoate	0.1490	0.0100	0.1300	0.0100	0.2100	0.7600
Ethyl 3-methylbutanoate	0.1090	0.1200	0.0600	0.0800	0.8900	0.3500
Ethyl 3-hydroxypropionate	0.0680	0.2700	0.0100	0.4900	0.0900	0.7500
Ethyl 3-hydroxybutanoate	0.0007	0.0000	0.0700	0.2800	0.0700	0.0100
Ethyl hexanoate	0.4180	0.0100	0.0000	0.1300	0.9700	0.5900
Ethyl 2-hydroxy-4-methylpentanoate	0.2780	0.0100	0.4500	0.5400	0.8000	0.3900
Ethyl 4-hydroxybutanoate	0.0680	0.0700	0.0000	0.1900	0.1400	0.1300
Diethyl butanedioate	0.0790	0.1200	0.6200	0.2210	0.2800	0.9500
Ethyl octanoate	0.8190	0.5900	0.4500	0.0600	0.2600	0.9400
Diethyle 2-hydroxypentanedioate	0.4710	0.0100	0.0000	0.1100	0.0900	0.3300
Ethyl decanoate	0.0600	0.0200	0.6500	0.5800	0.5800	0.2800
Ethyl 3-methyl butyl butanedioate	0.1570	0.2000	0.6400	0.1300	0.4200	0.1400
Ethyl 2-hydroxy-3-phenylpropanoate	0.0600	0.0700	0.8700	0.0700	0.3100	0.2000
SUM	0.5160	0.2600	0.5700	0.4200	0.3800	0.4600
Other fermentation volatile compounds						
Ethyl lactate	0.0210	0.3400	0.0400	0.6000	0.0900	0.5300

Isophenyl acetate	0.3670	0.3000	0.2300	0.0000	0.1600	0.0600
α Butyrolactone	0.1250	0.0800	0.3000	0.5700	0.3700	0.5400
Pantolactone	0.3160	0.0800	0.5800	0.0600	0.4200	0.6900
2-Phenylethanol	0.2300	0.0000	0.0100	0.0000	0.1100	0.1300
γ-Butyrolactone	0.4250	0.0100	0.1400	0.1200	0.0500	0.7700
Tartaric acid diethyl ester	0.0008	0.2100	0.0000	0.1800	0.1700	0.0900
2-Tert-butyl-5-propyl-1,3-dioxolan-4-one	0.2740	nd	nd	0.0100	0.8300	0.1600
3-Methylbutanol	0.1910	0.3000	0.8700	0.0100	0.4600	0.2600
2-Ethylbutanol	0.1340	0.6200	0.8200	0.6800	0.3100	0.8400
3,4-Dimethyl 2-hexanol	0.4900	0.0100	0.8400	0.7800	0.0600	0.4200
4-Methyl 1-pentanol	0.1800	0.0000	0.1900	0.0100	0.4300	0.0900
3-Methyl pentanol	0.0001	0.0000	0.0000	0.0000	0.0000	0.1400
2 Ethyl hexanol	0.3450	0.2600	0.0400	nd	0.4500	0.1600
Ethyl 2-hydroxyisovalerate	0.2330	0.1800	0.0200	0.0000	0.4000	0.4900
Diethyl malate	0.0007	0.0000	0.0200	0.0100	0.0100	0.1100
Diethyl succinate	0.0005	0.0000	0.0000	0.3800	0.1100	0.0500
Furaneol	nd	nd	nd	0.0300	nd	nd
2,6 Di (T-Butyl)-4-hydroxy-4-methyl-2,5 cyclohexadien-1-one	0.6580	0.3000	0.3600	0.3100	0.3200	0.8500
SUM	0.1910	0.0000	0.4600	0.0000	0.8500	0.2100
Fatty acids						
Heptanoic acid	0.0670	0.1800	0.4700	0.5100	0.6600	0.4100
Hexanoic acid	0.9300	0.0000	0.0700	0.2800	0.8100	0.7600
Octanoic acid	0.8500	0.6400	0.1800	0.3100	0.9000	0.8100
Isobutyric acid	0.0930	0.0800	0.1900	0.0200	0.1800	0.0500
Butanoic acid	nd	nd	nd	0.0500	nd	nd
3-Methylbutanoic acid	0.9380	0.5400	0.7700	0.0100	0.0200	0.7500
2-Methylbutanoic acid	0.3800	0.1000	0.7900	0.0000	0.3000	0.7300
2,4-Hexadienoic acid	0.8400	nd	0.8700	nd	0.1200	nd
9 Decenoic acid	0.0600	0.2600	0.0000	0.1200	0.1100	0.7100
SUM	0.9930	0.2400	0.6700	0.0600	0.6400	0.4700
Monoterpene						
β-Linalool	nd	nd	nd	0.0400	nd	nd
Phenolic esters						
Phenethyl acetate	0.0000	0.0000	0.2300	0.0100	0.9200	0.0300

General conclusion

This master's project aimed to understand the impact of berry stage of maturity on aromatic profile of white interspecific hybrid grapes and wines produced in Nova Scotia over two to meet following project hypothesis: 1) the maturity of berries favorably impacts the concentration of free and bound volatiles such as monoterpenes and FADP in the interspecific hybrid grape varieties by resulting in more favorable aroma profile in final wines, 2) warmer season in Nova Scotia positively influences the concentration of volatile compounds monoterpenes and C_{13}-norisoprenoids of berries and wines produced from white interspecific hybrids and 3) varietal volatile compounds present in hybrid grape varieties such as monoterpenes (linalool geraniol and nerol) and fatty acid degradation (hexanol, (Z)3-hexenol) products are strongly correlated with the presence of these compounds in wines.

The results obtained within the framework of this project made it possible to validate these hypotheses by demonstrating that higher accumulation of GDD (warmer seasons; year effect) and later maturity had positively impacted wine aroma composition in the studied interspecific hybrid *Vitis* varieties L'Acadie blanc, Seyval blanc and Osceolat Muscat grown in Nova Scotia. The results also allowed the discrimination of the interspecific hybrid cultivars based on their varietal character. Furthermore, clear relationships could be traced between the level of certain volatile compounds in grape and their concentration as free compounds in the wines.

Analysis of the aromatic profiles of grapes and wines from hybrid grape varieties has made it possible to reveal that the contents of certain compounds recognized for their favorable aromatic impact on wine (e.g.: terpenes and aromatic esters) compared with the levels previously reported in several varieties belonging to *V. vinifera.* The knowledge acquired on the volatile composition of these grape varieties and its relation to wines will make it possible to select suitable harvesting maturity and to select best oenological practices for hybrid grape varieties to optimize the aromatic quality of the wines produced.

To get an understanding about the relation between wine volatile composition and its impact on wine sensory properties, it would be interesting to investigate the sensory profiles of the produced wines in future. Since we used single yeast stain for fermentation, better to do additional research on finding the impact of mixed yeast strains on wine volatile composition. Furthermore, for better extraction of volatile compounds such as terpenes into wines, it would

be interesting to investigating the impact of skin maceration in white wines produced in Nova Scotia.

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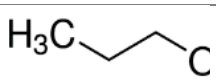
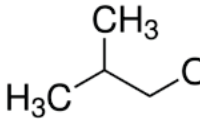
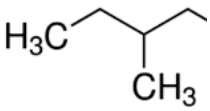
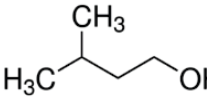
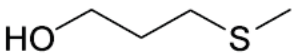
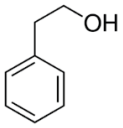
Annex 1 Wine secondary and tertiary aroma
2.4.2 Fermentation aroma

Higher alcohols

Total content of aroma compounds in wine is ranging from 0.8 -1.2 g/L, and 50% of that is higher alcohols making it quantitavely the largest group of aromatic compounds (Table 1.7). Alcohols are released into wine as secondary products of yeast metabolism and are mainly formed during the first two stages of alcoholic fermentation, via an anabolic pathway from glucose and a catabolic pathway from the corresponding amino acids (Chang, *et al*., 2014). The process by which amino acids are catabolized into higher alcohols is called the Ehrlich reaction, where amino acids are deaminated, *α*-keto- acids are decarboxylated and reduced to the correlating alcohol. The content in higher alcohol increases as the amino acids concentration in must increases (Anke, 2013). Ripening is positively related to the concentration in alcohols (Ubeda, *et al*., 2017).

Table 1.7: Structures and aroma descriptor of main higher alcohols found in wine.

Compound	Structure	Odor threshold value (µg/L)	Aroma descriptors
Propanol		306000	Alcoholic, fermented, fusel alcohol, tequila with a delicate fruity nuance of apple and pear (1)
Butanol		150 000 (b)	Fusel, spirituous (2)
2-Methyl-1- propanol		40,000 (b)	Solvent (3)
2-Methyl-1-butanol		65000	Nail polish (4)
3-methylbutan-1-ol		30,000 (b)	Pungent, alcohol (5)
3-(Methylthio) -1-propanol		4,500	Cooked vegetable (5)
2-Phenylethanol		14,000 (vs)	Sweet, floral, pink, honey (6)

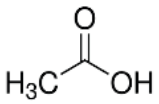
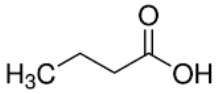
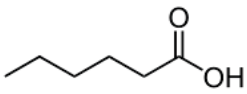
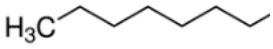
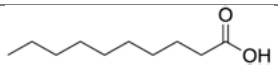
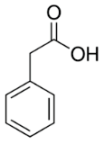
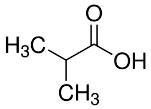
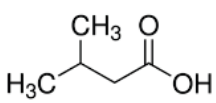
(b: threshold determined in hydroalkolic solution (10% ethanol, w / w) vs: threshold determined in synthetic wine)

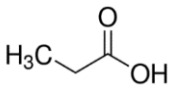
References [1](Anke, 2013), [2](Vilanova *et al.*, 2007), [3](Guth, 1997), [4](Bartowsky and Pretorius, 2009), [5](Chang, *et al*., 2014) and [6](Ferreira, *et al*., 2000).

Volatile acids

The volatile acid content of wine is usually between 500-1000 mg L^{-1} (10–15% of the total acid content) and about 90% is constituted of acetic acid (Bartowsky and Pretorius, 2009) (Table 1.8). Medium chain fatty acids (MCFA) are produced as intermediates from the biosynthesis of long-chain fatty acids during alcoholic fermentation by the yeasts and are produced during fatty acid biosynthesis from acetyl co-enzyme A (Bartowsky and Pretorius, 2009).

Table 1.8: Structures and aroma descriptor of main volatile acids found in wine.

Compound	Structure	Odor threshold value (µg/L)	Aroma descriptors
Acetic acid		200000 (at)	Volatile acidity, vinegar (1)
Butanoic acid		173 (b)	Cheese, rancid (2)
Hexanoic acid		3000	Cheese, rancid, fatty, sweaty (1)
Octanoic acid		500 (b)	Rancid, harsh, soapy, sweet, faint fruity, butter (3)
Decanoic acid		1000 (b)	Fatty, unpleasant, rancid, citrus, phenolic (3)
2-Phenylacetic acid		1000 (b)	Sweet, floral, honey, rose, chocolate, tobacco (2)
2-Methylpropanoic acid		2300 (b)	Cheese, rancid (3)
3-Methyl butanoic acid		33 (b)	Blue cheese (2)

Propionic acid		20000	Rancid, slightly pungent, vinegar (4)

(at: threshold determined in a hydroalkolic solution (10% ethanol) b: threshold determined in synthetic wine).

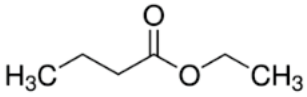
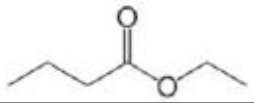
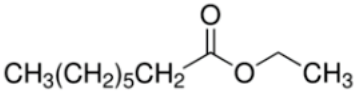
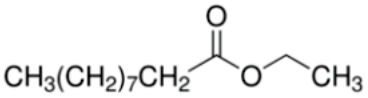
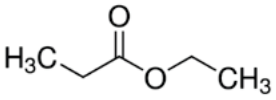
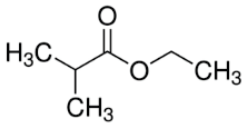
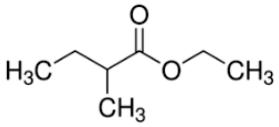
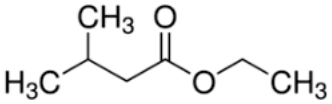
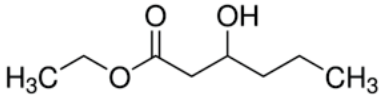
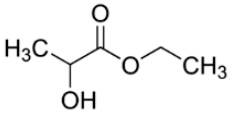
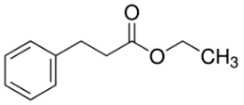
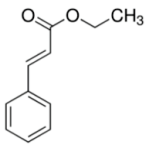
References [1](Guth, 1997), [2](Campo *et al.*, 2006), [3](Ferreira, *et al*., 2000) and [4](Anke, 2013).

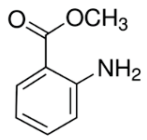
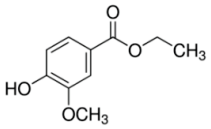
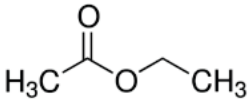
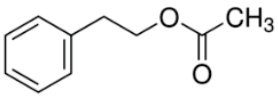
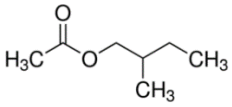
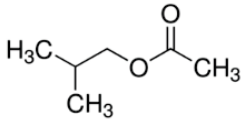
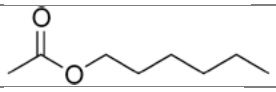
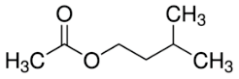
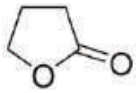
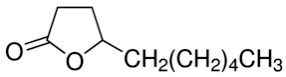
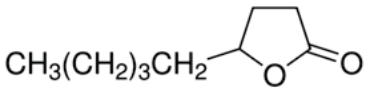
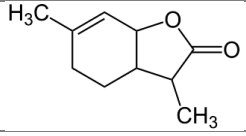
Esters

Esters are organic acids that are mainly enzymatically synthesized by yeast during alcoholic fermentation of wine and their contents can also be modulated by lactic acid bacteria during malolactic fermentation (Anke, 2013). Generally, in wine, esters are commonly attributed to imparting a "fruity" smell to wines. Esters can be divided into two groups: acetate esters and ethyl esters. Generally, acetate esters are present at higher concentrations than ethyl esters and are perceived as fruity aromas (Anke, 2013).

Ethyl esters are mainly produced by yeast metabolism through fatty acid acyl and acetyl Coenzyme A (CoA) pathways. During the biosynthesis of medium chain fatty acids, acyl-CoA intermediates formed are then esterified with ethanol by esterase and transferase enzymes, forming MCFA ethyl esters. Acetate esters, on the other hand, are produced through the condensation of yeast-derived higher alcohols with acetyl-CoA, again under the control of ester-forming enzymes (Lambrechts and Pretorius, 2018).
Grape-derived aliphatic alcohols and aldehydes are identified as precursors to acetate esters in wine. In particular, the C_6 compounds (*Trans*)-2-hexenal, hexanal, (*Trans*)-2-hexenol, and hexanol were shown to be precursors to hexyl acetate, whereas octanol and benzyl alcohol were identified as precursors to octyl acetate and benzyl acetate, respectively (Vilanova *et al.*, 2007) (Table 1.9). Ester concentrations differ among wine produced from different grape varieties, and there appears to be a synergy between the grape and the yeast metabolism in establishing the characteristic ester (Vilanova *et al.*, 2007). Quantity and category of esters are impacted by berry ripeness. For an example, hexyl acetate has been detected in post-fruit set samples and significantly increased early in berry development, followed by a significant drop at veraison (Styger, *et al*., 2011). According to Antalick *et al.*, (2015) 80% of esters measured in *Cabernet Sauvignon* and *Shiraz* grape cultivars were influenced by a varietal effect, whereas only 30% were influenced by grape maturity. The formation of esters also differs widely between yeast strains and other external factors such as fermentation temperature, nutrient availability, pH, unsaturated fatty acid/sterol levels, and oxygen levels play an important role in determining the end levels of esters in a wine (Styger, *et al*., 2011).

Table 1.9: Structure and aroma descriptors of esters, acetates and lactones found in wine.

Compound	Structure	Odor threshold value (µg/L)	Aroma descriptors
	Ethyl esters of fatty acids		
Ethyl butanoate		20	Floral, fruity (1)
Ethyl hexanoate		1	Green apple, banana, violets (2)
Ethyl octanoate		580	Pineapple, pear (3)
Ethyl decanoate		500	Floral, soap (3)
Ethyl propanoate		1800	Fruity (1)
Ethyl 2-methyl propanoate		0.1	Sweet, Fruity (2)
Ethyl 2-methyl butanoate		18	Sweet fruit (4)
Ethyl 3-methyl butanoate		3	Berry (4)
Ethyl 3-hydroxyhexanoate		45 (b)	Sweet, fruity, rubbery (5)
Ethyl lactate		14000	Strawberry (1)
Ethyl 2,3-dihydrocinnamate		1.6	Flowery, fruity (6)
Ethyl cinnamate		1.1	Cherry, plum, honey, cinnamon (6)

Methyl anthranilate		3	Fruit, grape (7)
Ethyl vanillate		990 (b)	Phenolic, burnt, smoked, metallic (4)
	Acetates		
Ethyl acetate		7500 (at)	Volatile acidity, solvent nail polish, fruity (8)
2-Phenylethyl acetate		250 (at)	Flowery, rose, honey,fruity (8)
3-Methyl butyl acetate		450	Banana, fruity (9)
2- Methyl propyl acetate		1600 (vs)	Banana, fruity (9)
Hexyl acetate		26	Sweet, perfume (4)
3-Methyl butyl acetate		30000	Banana (8)
	Lactones		
γ- Butyrolactone		100,000	Cream, oil, with nuances of bold (4)
γ- Decalactone		88	Fruity, peach, creamy and sweet with nuances of bold (10)
γ- Nonalactone		30	Sweet, creamy, coconut, fattywith nuances of butter (10)
Wine lactone		0.01 (b)	Coconut, spicy, sweet, dill (8)

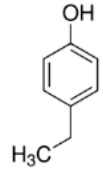
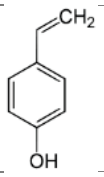
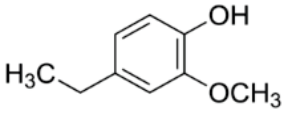
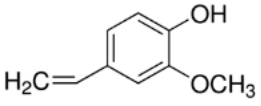
(at: threshold determined in a synthetic wine b: threshold determined in an alcoholic solution (10-14% ethanol v / v) vs: threshold determined in beer)

References [1](Bartowsky and Pretorius, 2009), [2](Maturano *et al.*, 2018), [3](Anke, 2013), [4](Slegers *et al.*, 2015), [5](Zea *et al.*, 2007), [6](Ferreira, *et al.*, 2000), [7](Aubry *et al.*, 1997), [8](Guth, 1997), [9](Guth, 1999), [10](Campo *et al.*, 2006).

Volatile phenols

The origin of volatile phenols involves sequential action of two enzymes on a hydroxycinnamic acid (ferulic, *p*-coumaric or caffeic acid) substrate, which are naturally found in grape musts (Teixeira *et al.*, 2015). Volatile phenols are very important in term of wine sensory characteristics, because at elevated concentrations they are associated with an unpleasant aroma (Ur *et al.*, 2016) whereas at low concentrations, they provide a certain aromatic complexity (leather notes, spices, smoke) (Table 1.10).

Table 1.10: Structure and aroma descriptors of some volatile phenols found in wine.

Compound	Structure	Odor threshold value (µg/L)	Aroma descriptor
4-Ethylphenol		440	Medicine, stable (1)
4-Vinylphenol		180	Pharmaceutical (1)
4-Ethylguaiacol		33 (b)	Phenolic, sweet (2)
4-Vinylguaiacol		10,000 (vs)	Clove like, phenolic (2)

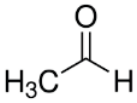
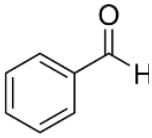
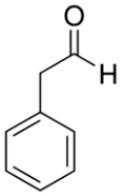
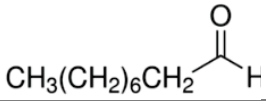
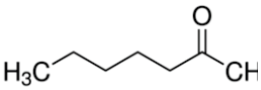
(b: threshold determined in an alkaline solution (10% ethanol) vs: threshold determined in water)

References [1](Campo *et al.*, 2006) and [2] (Bartowsky and Pretorius, 2009).

Carbonyl compound

The most prominent carbonyl compound detected in wine is acetaldehyde and present at levels ranging from 10 to 300 mg/L. Acetaldehyde perception has a sensory threshold value of 100 mg/L in wine (Anke, 2013). Carbonyl compounds are generally associated with nutty and even citrus aroma and are usually of interest due to their low threshold values(Wei *et al.*, 2019) (Table 1.11).

Table 1.11: Structure and aroma descriptors of some carbonyl compounds found in wine.

Compound	Structure	Odor threshold value (µg/L)	Aroma descriptor
Acetaldehyde		100000	Sour, green apple (1)
Benzaldehyde		375	Bitter almond (2)
Phenylacetaldehyde		1 (b)	Honey, pink, powdery, chocolate with subtle nuances earthy (3)
Nonanal		1 (vs)	Wax, aldehyde, citrus, with a hint of lime zest, cucumber (4)
2-Heptanone		140-1330 (vs)	Cheese, fruity, ketone, green banana with nuance creamy (2)

(b: threshold determined in a synthetic wine vs: threshold determined in water)

References [1](Anke, 2013), [2](Maturano *et al.*, 2018), [3](Campo et al., 2006) and [4](Buttery, *et al.*, 1988).

2.4.3 Aromas from wine ageing

The composition of wine changes continuously during storage as a function of the combined influence of storage temperature, oxygen content and storage time (Hernanz *et al.*, 2009). During wine ageing many reactions occur that cause significant effect on organoleptic properties of wine. The most obvious change being wine colour, which refers to a change in the phenol profile (Kalkan and Dündar, 2017). During storage, the total volatile concentration decreased progressively mainly due to the loss of alcohols. Results by Hernanz *et al.* (2009) had shown that the levels of carbonyl compounds have been (acetaldehyde, furfural and 5-

hydroxymethyl furfural) decreased while the concentration of acids and esters has been increased in *Zalema* and *Colombard* wines in Spain.

Oxidation plays an important role in volatile and nonvolatile concentrations in bottle-aged wines as it causes the conversion of ethanol into acetaldehyde, and its conjugates with tannins or anthocyanins (Lambropoulos and Roussis, 2007). This phenomenon results in an oxidative aroma, and disappearance of fruity and flavours produced during fermentation (Liu *et al.*, 2016) (Styger, *et al*., 2011). (Table 1.12).

Table 1.12: Chemical compounds implicated in aroma evolution during wine ageing.

Compounds	Examples	Trend during ageing	Reference
Polyfunctional thiols	3SH, 3SHA, 4SMP, benzyl mercaptan	Decrease Possible increase	(1)
H2S		Possible increase	(2), (3)
MeSH		Possible increase	(2)
DMS		Increase	(3)
C3−C10 fatty acid esters	ethyl hexanoate	Decrease	(4)
Ethyl acetate		Increase	(4)
Acetate esters	3-methylbutyl acetate	Decrease	(2)
Branched-chain ethyl esters	ethyl 3-methylbutanoat	Increase	(2)
Acetaldehyde		Increase	(5)
Aliphatic aldehydes	trans-2-nonenal	Increase	(6)
Higher alcohols	3-methyl-1-butanol	Generally stable	(6)
Strecker aldehydes	methional	Increase	(6)
Sotolon		Increase	(2)
3-methyl-2,4-nonadione		Increase	(1)
Aliphatic lactones	nonalactone	Increase	(1)
Norisoprenoids	TDN, damascenone	Increase	(2)
Monoterpenes	linalool	Increase and then decrease	(2)
Methoxypyrazines	3-isobutyl-2-methoxypyrazine	Decrease	(7)

References [1](Ugliano, 2013), [2](Moio *et al.*, 2004), [3](Ugliano *et al.*, 2012), [4](Liu *et al.*, 2016), [5](Kreitman, *et al*., 2013), [6](Escudero *et al.*, 2000) and [7](Blake *et al.*, 2009).

The amount of the branched fatty acid ethyl esters is more or less stable, or can increase during the ageing of wine. In *Muscadet* wines from different vintages, the levels of the branched esters increased and the straight-chain esters decreased with ageing (Chneider, 2005). According to the findings of Liu *et al.*, (2016), volatile compounds such as esters, alcohols, and benzonoids, such as diethyl succinate, hexyl acetate, isoamyl alcohol, 1-nonaol,

benzaldehyde, benzyl ethyl aldehyde, and phenol decrease with ageing. Similar trend was observed for most of terpenes and C_{13}-norisoprenoids, such as linalool, *β*-citronellol, *β*-damascenone, and 6-methyl-5-hepten-2-one. In detail, norisoprenoids exhibited a decreasing trend during bottle ageing, whereas terpenes showed an increasing and then decreasing trend (Liu *et al.*, (2016). Nonanal, decanal, and other carbonyl compounds such as acetoin, 2, 6-dimethyl-4-heptanone, γ-butyrolactone, and furfural showed an increase in concentration in the early storage period and then displayed a decrease (Liu *et al.*, 2016). In Spanish red wines, phenol aldehydes [vanillin (4-hydroxy- 3-methoxybenzaldehyde) and syring aldehyde (4-hydroxy-3,5- dimethoxybenzaldehyde)], volatile phenols (2-methoxyphenol, 2-methoxy-4-methylphenol, and 4-allyl-2-methoxyphenol), and some furanic compounds depicted a decreasing trend during the first years of storage in the bottle, while generating 4-ethyl phenol, 2-methoxy-4- ethyl phenol, 2-furaldehyde, and whisky lactone (Ollin, 2009). Over time, changes can occur due to esterification and hydrolysis processes as wines re-establish equilibrium between the esters, alcohols, and acids present immediately after fermentation in forming wine aged aroma (Blake *et al.*, 2009).

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Annex 2 Poster presented at the Proceedings of Center SÈVE 2020 student scientific poster competition

Summary

Due to its proximity with the Atlantic Ocean, Maritime Provinces such as Nova Scotia show highly variable climatic conditions that can lead to different levels of berry ripeness and quality. We hypothesized that GDD accumulation over time has a significant positive impact on grape physicochemical parameters (sugars and acids) thus affects sensory scores of wine. In the current study, three hybrid grape varieties (L'Acadie blanc, Seyval blanc, Osceola muscat) harvested at three different physiological stages with different accumulated GDD were analyzed for yield, crop load, grape physiological parameters and must physicochemical composition. Despite of the cultivar, with berry ripening, the level of TSS significantly increased ($P<0.05$) and acidity significantly decreased. And grapes harvested at stage E-L 39 (935.1 GDD in 2019), showed the highest TSS of 18.8, 18.56 and 19.6 for three cultivars OM, SB and AC respectively. In year 2020, GDD accumulation was faster than year 2019, thus had produced grapes with higher TSS, and lower acidity at the same harvesting stage as year 2019. Thus, the stage of grape harvesting is important for the quality of wine, and grapes harvested with higher accumulated GDD are more appealing for wine making with better sensory appeal.

Reference

Effect of accumulated GDD on the ripening of three hybrid grape cultivars *(*L'Acadie blanc, Seyval blanc, Osceola muscat*)* grown in Nova Scotia, *Proceedings of Center SÈVE 2020 student scientific poster competition* November 25 and 26, 2020. pp. 18-19 (No 36).

Poster

Effect of accumulated GDD on the ripening of three hybrid grape cultivars (*L'Acadie blanc, Seyval blanc, Osceola muscat*) grown in Nova Scotia

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Introduction

Background

Due to its proximity with the Atlantic Ocean, Maritime Provinces such as Nova Scotia shows a yearly variation in climatic conditions that can lead to different levels of berry ripeness and quality (Jantzi and Mcsweeney, 2019). Heat accumulation, as measured in growing-degree days (GDD, based on 10 °C), has a significant impact on berry ripening and quality. To ensure quality grape production year after year, most NS growers produce interspecific hybrid varieties that show higher tolerance to abiotic and biotic stresses. Harvest date which is also influenced by GDD has a great impact on the chemical composition of grapes, allowing grapes to develop varietal characteristics (Pedneault et al., 2013). Changes arise are both physical (e.g., yield, berry weight) and chemical (e.g., pH, sugars, acidity).

Objectives

Understand the impact of different patterns of growing-degree days accumulation on fruit composition of *Vitis* sp. berries in Nova scotia at different ripening stages.

Methodology

Experimental design

White interspecific hybrid grape varieties Osceola Muscat, Seyval Blanc and l' Acadie blanc were harvested in a commercial vineyard located in the Annapolis Valley, NS, according to a completely randomized design, during the 2019 and 2020 seasons. Berries were picked at three maturity stages corresponding to phenological stages EL-37, EL-38 and EL-39 from the Lorenz scale for grapevine phenological stages (Lorenz et al., 1995). Experimental unit consisted of 20 plants where 5 to 6 clusters were randomly selected, for a total of 1-2 kg per sample.

Figure 1. Sampling site (NS) and varieties studied Oscéolat Muscat (A), Seyval blanc (B), L'Acadie blanc (C).

Berry physiological and physicochemical analysis

- Cluster weight of 5 randomly selected clusters, weight of 50 randomly selected berries from 5 clusters and crop load per plant were determined in all three cultivars.
- 50 randomly selected berries were manually crushed and juices were extracted by pressing.
- Extracted juice was analyzed for total soluble solid content (TSS), pH, titratable acidity (TA), yeast assimilable nitrogen (YAN) and primary ammonia nitrogen (PAN).
- TSS, pH and TA were analyzed with the use of a digital hand refractometer (PAL-1), pH meter (MP 220) and titratable acidity mini titrator (HI 84502) respectively. YAN and PAN was measured using Unitab AMM 150 Reagent Kit and the absorbance was measured using UV visible spectrophotometer (340 nm).

Statistical analysis

Analysis of variance and tests for normality and data homogeneity were carried out using the mixed procedure of the SAS software. Means were compared using a Tukey's test (P = 0.05).

Results

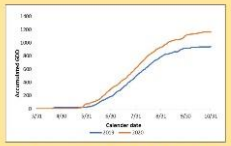

Figure 1: Rate of GDD accumulation (based on 10 degree C) on our experimental site located in Annapolis Valley, NS, in 2019 and 2020, from April 1 to October 31.

Results

Table 1 : Variation of crop load per plant for three hybrid grape *cultivars Osceola muscat, Seyval blanc and L'Acadie blanc* grown in our experimental site in Nova Scotia for two consecutive years 2019 and 2020. Values followed by different letters are significantly different according to Tukey's test at P ≤ 0.05

Cultivar	Physiological parameters	2019 (GDD on last harvest: 935)	2020 (GDD on last harvest: 1132)
Osceola Muscat	Berry weight (g)	1.66 ± 0.07 ^{a}	1.66 ± 0.04 ^{a}
	Cluster weight (g)	127.02 ± 43.4^{a}	72.18 ± 15.2 ^{b}
	Crop load (kg/plant)	3.45 ± 0.2 ^{a}	3.46 ± 0.43 ^{a}
Seyval blanc	Berry weight (g)	1.63 ± 0.13 ^{a}	1.66 ± 0.11 ^{a}
	Cluster weight (g)	208.56 ± 66.2^{a}	322.28 ± 63.4^{a}
	Crop load (kg/plant)	1.07 ± 0.5 ^{b}	2.03 ± 0.18 ^{b}
L' Acadie blanc	Berry weight (g)	1.13 ± 0.09 ^{a}	1.12 ± 0.02 ^{a}
	Cluster weight (g)	116.4 ± 8.6 ^{a}	102.45 ± 6.7 ^{b}
	Crop load (kg/plant)	1.89 ± 0.22 ^{a}	3.28 ± 0.48 ^{b}

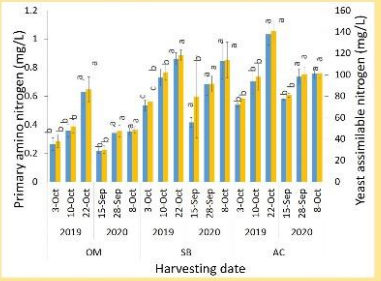

Figure 4: Variation of Primary amino nitrogen (PAN) and yeast assimilable nitrogen (YAN) of three hybrid grape *cultivars Osceola muscat, L'Acadie blanc and Seyval blanc* grown in Nova Scotia in two consecutive years 2019 and 2020. Each consecutive data point correspond to phenological stages EL-37, EL-38 and EL-39. For a given year, bars followed by different letters are significantly different according to Tukey's test at p ≤ 0.05.

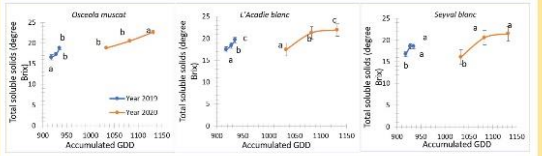

Figure 2: Variation of Total soluble solid content (TSS) of three hybrid grape *cultivars Osceola muscat, L'Acadie blanc and Seyval blanc* grown in our experimental site in Nova Scotia in two consecutive years 2019 (blue) and 2020 (orange). Each consecutive data point correspond to phenological stages EL-37, EL-38 and EL-39. For a given curve, points followed by different letters are significantly different according to Tukey's test at p ≤ 0.05.

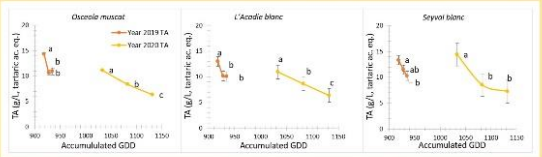

Figure 3: Variation of Titratable acidity (TA) of three hybrid grape *cultivars Osceola muscat , L'Acadie blanc and Seyval blanc* grown in Nova Scotia in two consecutive years 2019 (orange) and 2020 (yellow). Each consecutive data point correspond to phenological stages EL-37, EL-38 and EL-39. For a given curve, points followed by different letters are significantly different according to Tukey's test at p ≤ 0.05.

Discussion and Conclusion

- Higher rate of heat accumulation in 2020 influenced berry ripening and physiology.
- Crop load of *Seyval blanc* and *L'Acadie blanc* significantly differed yearly and year 2020 had depicted a comparatively higher crop load than 2019. Faster accumulation of GDD had a positive impact on berry yield in 2020.
- Despite of the cultivar, TSS significantly increased and acidity significantly decreased with berry ripening.
- At the same phenological stages, 2020 berries showed a higher TSS and a lower acidity compared those from 2019.
- YAN and PAN of three cultivars slightly increased during ripening, which is directly affected by nutritional status of vineyards.
- 2019 and 2020 showed very different conditions and thus provided an extended range of climate that may arise and affect grape production in Nova Scotia. Given the TSS and TA measured during this study, winemakers should be flexible about the wine style that they can produce from Seyval blanc, L'Acadie blanc and Osceola Muscat.

Acknowledgement

This project was funded by Canadian Vine Certification Network (RCCV), Agriculture and Agri-Food Canada and the Nova Scotia Department of Agriculture.
Special thanks for all the members of Luckett vineyards, gaspereau valley, Nova scotia and all the members of laboratory of Department of science, Saint Anne University, Nova Scotia.

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Annex 3 Poster presented at 45th ASEV-Eastern Section Virtual Conference

Summary

Nova Scotia shows variable yearly climatic conditions making it a challenging environment for grape production. Many factors affect the quality of the grapes and its final product, wine, including geographical origin (e.g., terroir), grape cultivar and berry ripening. In the current study, three *Vitis* varieties L'Acadie blanc, Seyval blanc and Osceola Muscat were harvested at three different phenological stages (EL-37, EL-38, EL-39, based on the Lorenz scale of *Vitis* sp. phenology), corresponding to different counts of GDD. Physiochemical data of berries (berry weight, cluster weight, crop load, total soluble solids (TSS), pH, titratable acidity (TA) and chemical composition of wine (TSS, TA, free volatile composition) were analyzed.

Fermentation-related volatile compounds (free fatty acids, fatty acid ethyl esters and higher alcohols) accounted for the highest proportion of wine volatile compared to variety-related volatile such as C_6 alcohols, terpenes, C_{13}-norisoprenoids and volatile phenols. 3-Methyl-1-butanol was the main aroma compound found in wine from all analyzed cultivars, followed by 2-phenylethanol which showed a significant increase from EL-37 to EL-39 in all wines. Similar trend was observed in other fermentation-related compounds such as ethyl lactate, isobutyl acetate, α-butyrolactone, pantolactone whose concentration significantly increased with ripening. On the other hand, the concentration of fatty acid degradation products such as cis 3-hexanol and 1-hexanol significantly decreased with ripening. Aromatic esters such as phenyl ethyl acetates were detected at very low levels in wine, but significantly increased by 166% from stage EL-37 to EL-9.

Reference

K. Lakmali, M. Dorais, K. Pedneault. Impact of Harvest Date on the Chemical Composition of Wines Produced from Interspecific Vitis sp. Cultivars Grown in Nova Scotia, Canada over two seasons. *45th ASEV-Eastern Section Annual Virtual Conference*. 7-8 july 2021, online.

Poster

Impact of harvest date on the chemical composition of wines produced from interspecific *Vitis* sp. cultivars grown in Nova Scotia, Canada over two seasons

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Introduction

Cold climate grape production occurs between last Spring and first Fall frosts, making cold climate wine quality largely dependent on the climate condition to properly ripen berries (Pedneault et al., 2013). Hence, in cold climate, berries are often harvested earlier than anticipated, due to quick temperature changes in Fall. However, wine quality largely relies on grape ripeness for basic parameters (acidity, alcohol content, etc.) and varietal aroma (Plessis & Rooyen, 1982). The relationship between berry ripeness and wine chemical composition has been studied in *Vitis vinifera* cultivars (Gao et al., 2019) but little investigated in interspecific hybrid *Vitis* sp. grown in northern areas such as Nova Scotia. In this context, this project aimed at investigating the composition of *L'Acadie blanc*, *Osceola Muscat* and *Seyval blanc* wines made from berries harvested at three ripening stages in Nova Scotia, Canada during the 2019 and the 2020 seasons.

Methodology

Experimental design and sampling

White interspecific hybrid grape varieties *Osceola Muscat*, *Seyval blanc* and *L'Acadie blanc* were harvested in a commercial vineyard located in the Gaspereau Valley, NS, according to a completely randomized design, during the 2019 and 2020 seasons. Samples (30 to 60 kg of berries per sample) were picked at three phenological stages EL-37, EL-38 and EL-39 (based on the EL scale for grapevine phenology; Lorenz *et al.*, 1995), corresponding to 918, 928, 935 growing-degree days (GDD) in 2019 and 1034, 1083 and 1132 GDD in 2020. Each experimental unit included four panels (separated sections over a row in vineyard) consist of 4 to 5 grapevines per panel. Each winemaking sample consisted of 30 to 40 kg of grapes and were sulfited until winemaking.

Five to six clusters per sample were put aside for juice measurements (total soluble solid, pH, titratable acidity (TA), yeast assimilable nitrogen and primary ammonia nitrogen.

Winemaking

Berries were processed into wine shortly after harvest, using the winemaking protocol described by Comfort & Johnson (2009). In short, berries were destemmed, crushed and pressed. Fermentation was started at 18 °C using Saccharomyces cerevisiae after adjusting the sulphate level to 1 mL/L wine using a potassium metabisulfite solution at 40 mg/L. Alcoholic fermentation were carried out in 5 to 7 days. Wines were then racked, clarified, cold stabilized, filtered, bottled and kept for aging at 4 °C.

Figure 1. Fermentation room at the research winery of Université Saint-Anne.

Wine chemical analysis

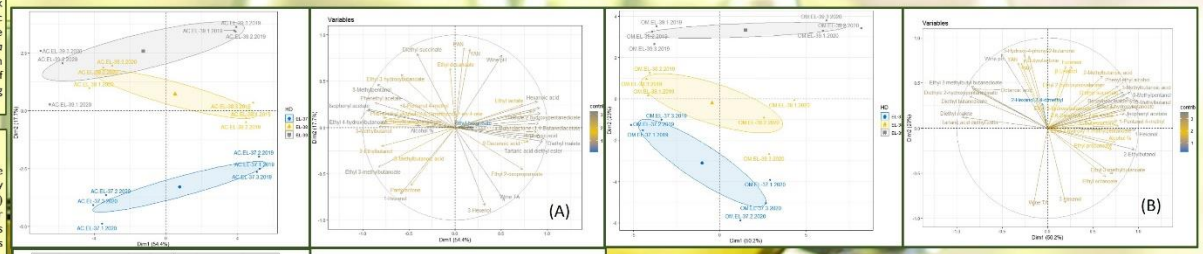
Wine TA and pH was measured using Hanna digital mini titrator and digital pH meter.

Wine volatiles were extracted by solid phase extraction (Lopez et al., 2002), concentrated and analyzed on an Agilent 7890A (Santa Clara CA, USA) gas chromatograph coupled to a flame ionization detector and a mass spectrometer (GC-MS-FID), on an HP-5MS Ultra inert column (30 m×250 µm×0.25 µm, Santa Clara CA, USA). Peaks were identified using authentic standards and the NIST Mass spectral library, and quantified against 2-Octanol (internal standard) using the FID signal.

Statistical analysis

Analysis of variance and tests for normality and data homogeneity were carried out using the mixed procedure of the SAS version 7.0 software (Caray, NC, USA). Means were compared using a Tukey's test (P = 0.05). Principal component analysis were carried out using R Studio (Boca Raton, FL, USA).

Results



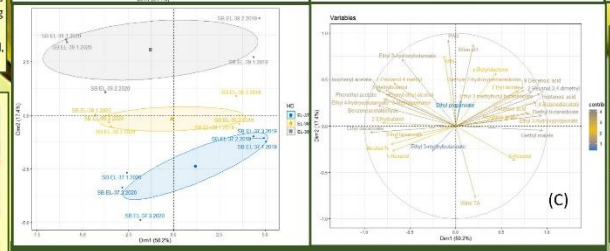


Figure 2. Principal component analysis (PCA) of wine chemical composition, including free volatiles compounds (39 compounds), juice primary amino nitrogen (PAN) and yeast assimilable nitrogen (YAN) content, wine pH, titratable acidity (TA) and alcohol content (alcohol %) of *L'Acadie blanc* (AC; A), *Osceola Muscat* (OM; B) and *Seyval blanc* (SB; C) harvested at the phenological stages EL-37, EL-38 and EL-39 in Nova Scotia, in years 2019 and 2020. Samples are plotted on the right graphs whereas variables are plots on the left graphs.
(Colour scale from blue-yellow –ash represent the contribution from each parameter towards the model and each point in the left plot represent the combination of Cultivar, Phenological stage, Replicate and Year)

Conclusion

- In all three cultivars AC, OM and SB there is a proper separation of variables according to the harvesting date and year.
- Despite of the cultivar, wine TA significantly decreased with berry ripening.
- For each variety, wine samples from the corresponding phenological stages had a lower acidity in 2020 when compared to those from 2019.
- Grape ripening stage significantly impacted the accumulation of wine volatiles as Aromatic esters (Phenethyl acetate), Monoterpenes (β-Linalool), Fatty acid ethyl esters, Furaneol and γ- Butyrolactone.
- *Osceola Muscat* wine produced during the warmer 2020 season had more monoterpenes (β-Linalool) and furaneol from *Osceola Muscat* compared to those from 2019. In addition, those compounds were not detected in wines from other cultivars, suggesting they might contribute to the varietal aroma of *Osceola Muscat* wine.
- Within challenging climatic conditions, higher accumulation of GDD (warmer season) positively impacted berry ripening at all phenological stages in all studied interspecific hybrid *Vitis* varieties.

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