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Tecniche di concimazione azotata frazionata per il mantenimento dell'acidità nella varietà Glera in un ambiente del nord est Italia (DOCProsecco)

Introduction

Grapes and wines quality and yield are the two main factors to consider to assess the success of a growing season. While yield means the baseline for a grape growers salary, quality allows access to bonus payment, obtain the protected denomination of origin (PDO) labelling, and raise the quotation of his production. Concerning grapes and wines, quality consists of the concentration of many chemicals compound, the overall physicochemical traits, and typical aroma and flavours.

In the Italian landscape, all the above features cited features are normed towards each producer who wants to access the PDO labelling, which certifies his product's quality. To ensure the best quality in each wine is essential to harvest grapes at the right time, which means at the best compromise among technological, phenolic and aromatic ripeness.

In sparkling wines, sugar and acid ratio and total titratable acidity (TTA) are two essential technological factors. At the same time, aromatic ripeness gives all varietal aroma and pleasant odoriferous compounds to the wine. (Carlos et al. 2007) (Traite d'enologie, 2013). Italy, France, Germany, and Spain are the most producers of sparkling wines worldwide. This market has continuously increased since last two decads ("OIV - Organizzazione Internazionale Della Vigna e Del Vino" n.d.). In this case, quality is a crucial factor to preserve the producer's competitiveness in the worldwide trade of this category of wine.

Since the last decads, grapes ripeness balance has been compromised by climate change (CC). Moreover, CC are evolving faster in the Mediterranean basin than in the rest of the world. Since the last century, mean temperatures have risen by 1.4°C, 0.4°C more than the global average. In 2040 and 2100, the average temperature could increase by 2.2 and 6°C, respectively ("Home - Berkeley Earth" n.d.). Ramos et al., 2008 and Van Leeuwen et al., 2019 stated that grapevine average growing season temperature (AGST) (temperature recorded from April to October) had increased about 2°C in Spain Alsace region. That means temperature increasing could affect more summer crops, like vines, than expected. Although worldwide precipitations are decreasing ("Home - Berkeley Earth" n.d.), in the Mediterranean region, total rainfall does not have a clear trend changing the distribution among seasons (de Luis et al. 2009). Indeed, extreme climate events, such as drought, heat waves, heavy precipitation, and flood, are more frequent (Bartolini et al. 2014; Klein Tank and Können 2003).

The main effect of climate warming towards grapevines is the decoupling between grapes ripeness component (Poni et al. 2018; Van Leeuwen and Destrac-Irvine 2017). Higher temperatures accelerate grapes ripening, as a consequence sugars content increase, whereas organic acids concentration decrease in both grapes and must (Keller 2010; Lakso and Kliewer 1978). Hence, in warmer climate wines tend to contain more alcohol (Duchêne and Schneider 2005; van Leeuwen et al. 2019; Van Leeuwen and Destrac-Irvine 2017) and less TTA (van Leeuwen et al. 2019).

TTA is the sum of all the organic acids present in grapes and wines. Tartaric acid (TA) and malic acids (MA) are the most abundant. Leaves produce TA from post-anthesis to veraison, while also green berries can produce MA. These acids are stored in fruit cell vacuoles. TA concentration doesn't change, but MA is converted into fructose and glucose as an energy source at the onset of ripening (Keller 2010; Carlos et al. 2007). There is a strong negative correlation between MA and temperature affecting MA synthesis and catabolism (Carlos et al. 2007). Acids play an essential role in moulding the wine taste, maintaining good pH values (3÷4), and ensuring stability and longevity (Carlos et al. 2007). As a fact, the selection of *Vitis vinifera* cultivar and clones with higher values of TA may provide a higher and stable level of TTA in wines (Poni et al. 2018).

Furthermore, grapevine phenology stages are developing faster in the last two decades (Mira de Orduña 2010; Ruml 2021; Webb et al. 2012). Consequently, grapes ripeness happened days and weeks before in many varieties (Ruml 2021; Webb et al. 2012; Jones and Alves 2012; Ramos 2017; van Leeuwen et al. 2019; Van Leeuwen and Destrac-Irvine 2017).

To avoid too much alcohol and preserve the acidity in wines, grape growers tend to harvest grapes early, days before than the complete aromatic ripeness. In this case, wines appear not entirely mature and contain unpleasant veggie-green aromas (Poni et al. 2018; Alberto Palliotti et al. 2014). In other cases, the advance of grapes maturity in the warmer period led to obtaining less aromatic wines containing smooth and cooked aromas (Duchêne and Schneider 2005; van Leeuwen et al. 2019; Van Leeuwen and Destrac-Irvine 2017).

Many authors propose different ways to delay grapes ripeness and enhance vines quality despite CC issues.

Different clones among the same varieties showed variations among sugars concentration in berries (van Leeuwen et al. 2012; de Lorenzis et al. 2017). Instead, TTA seems to be strictly related to the rootstock choice (Duchêne et al. 2014; W. M. Kliewer 1967). However, in the XXth century, the selection of early ripening and high sugars production promoted *Vitis vinifera* varieties and clones with opposite criteria than current requirements (Van Leeuwen and Destrac-Irvine 2017). Results from new clones selection may appear only in the next future. A significant difference in both sugar and acidity (W. M. Kliewer 1967; Liu et al. 2006) level was found between *Vitis vinifera* varieties, as well as the difference in ripening date: 60days between the earliest and the latest cultivar (Van Leeuwen and Destrac-Irvine 2017). This means early ripen cultivar could replace later ones. Nevertheless, the import of non-local varieties could affect the typicity and the terroir features of the wines (Van Leeuwen and Destrac-Irvine 2017). Moving towards a cooler climate through latitude and altitude means changing in weather and sunlight conditions affecting quality and aroma in wines (Spayd et al. 2002). Moreover, setting new vineyards may have detrimental environmental effects disturbing the wildlife habitat and ecosystem (Hannah et al. 2013). Concerning agronomic practices, is noted the relationship between yield and quality (Poni et al. 2004; Howell 2001). Moving from low to high yield production has verified a decrease of total soluble solids (TSS) and TTA, while wines' aroma becomes from fruity to veggie (Roby and Matthews 2004). Water supply deficit at pre-veraison may produce variation in phenological stages development and an increase in TSS into berries. On the other hand, water deficit at post-veraison led to must components concentration due to berries drying. It has been proven late pruning will delay budbreak, (FRIEND and TROUGHT 2007). The chosen training system with higher trunk to increase the distance between soil and vegetation can reduce the microclimate temperature into the vegetation (Reynolds and Vanden Heuvel 2009), because in summer the air layer nearest to the soil surface is warmer than farther ones. Leaf area reducing has a direct effect on sugar synthesis, without affect acid accumulation (Parker et al. 2015). At the same time, it could increase herbaceous aroma (W. Mark Kliewer and Dokoozlian 2005).

Many authors verified the clear effect of nitrogen fertilisation on delaying of grapes ripening, reduction of TSS and increase of TTA (Baiano et al. 2011; Delgado et al. 2004; Thomidis et al. 2016). These could be indirect consequences of nitrogen fertilisation linked to a vigour increasing (Poni et al. 2018; Etienne et al. 2013). Vigour plants have more shoots and leaves, reducing light penetration and altering canopy microclimate. Nitrogen availability also has a direct effect on grapes and wine quality. More yeast available nitrogen (YAN) increase yeast activity during fermentation improving wines quality (BELL and HENSCHKE 2005). N fertilisation effect positively also the aromatic potential of wines increasing the free amino acids (FAA) concentrations and aromatic compounds precursors (Webster et al. 1993; Choné et al. 2006; Canoura, Kelly, and Ojeda 2018; Jiménez-Moreno et al. 2020; Hernández-Orte, Cacho, and Ferreira 2002; Bouzas-Cid et al. 2015; Lee and Schreiner 2010). FAA. role in wine aroma as a matter of fact (Trigo-Córdoba et al. 2014; Roda et al. 2019; Carlos et al. 2007; Hernández-Orte, Cacho, and Ferreira 2002) explained by FAA conversion into higher alcohols and phenolic during the alcoholic fermentation (Keller 2010; Carlos et al. 2007). In particular, threonine, phenylalanine and aspartic acid seem to have a key role during must fermentation and wine aroma (Hernández-Orte, Cacho, and Ferreira 2002; Choné et al. 2006), whereas most abundant FAA on must are arginine and proline, from 60 to 70% of total FAA (Keller 2010).

Each technic mentioned above cannot face CC alone, but the combination of many strategies may help delay grapes ripening and preserve wines quality nowadays (van Leeuwen et al. 2019).

This study aimed to verify the positive effect of additional nitrogen fertilisation in berries' biochemicals, improve the aromatic profile, and raise the TTA in the wine. Furthermore, the FAA profile of ripening berries of both control and fertiliser treatments has been analysed to test their connection to the improve of aroma in sparkling wines. Finally, the experiment proved the procedure exploited for vines additional fertilisation didn't affect vines vigour and yield. Nitrogen accumulation into fruits seems to reach the higher level when N fertiliser is provided from blooming to veraisnon (Cheng, Lakso, and Goffinet 2007). Moreover, nitrate (NO_3^-) enhances organics acids synthesis instead of ammonium (NH_4^+) (BENZIONI, VAADIA, and LIPS 1971; Smith and Raven 1979; Scheible et al. 1997).

Materials and Method

Experimental site and vine management.

The trials took place in northeast Italy, in the middle of the Prosecco PDO area, Veneto region (45.806259°N; 12.343800°E) in the winery "Azienda c/o Vazzola" during 2018, 2019, and 2020 growing seasons. This geographic region is called "High Plain". It is a floodplain rich in sand and stones. The annual and summer historic mean temperatures record 13.1°C and 21.9°C, respectively, while the annual and summer mean rainfall records 1155mm and 262mm. The altitude of the location is around 40 m above sea level. Climate data was recorded by the nearest ARPAV weather station, less than 1Km from the experimental site. The 1 ha vineyard was planted in 2010 to white grape Glera (clone ISV19) grafted on Kober5bb rootstock, with a vine and row spacing of 1.20m and 2.80m, respectively. The planting density results in 2976 vines per hectare. The vines are trained in a vertical position, using a double Guyot trellis system. The two main canes are bent like arches towards the ground, it is a typical training system for Glera in the Veneto region, called "Cappuccina". The vineyard is cultivated following integrated diseases and pests management. The irrigation system consists of a drip line located 30cm underground. Budbreak usually happens in April, whereas grapes are harvested from the end of August to the middle of September. Grapes from this area are assigned to the Prosecco sparkling wine production. The farmers have to respect many quality parameters of grapes to obtain PDO labelling, for example total titratable acidity, aroma and flavour are some main parameters.

Experimental design.

The vineyard has been split into two plots, each one per treatment. Six consecutive rows, three per plot, have been chosen to avoid spatial and soil interferences during the sampling. The control received 35kg/ha of nitrogen from post-harvest to full flowering fractioned in two operations with mineral fertiliser. The treatment thesis received 15kg/ha of nitrogen post-harvest spreading the same fertiliser for the winter fertilization of the control thesis. In addition, the treated thesis received 13g/vine of nitrogen, split in three operations between flowering and veraison using the existing irrigation plan. Thanks to fertigation, 38kg/ha of nitrogen were spread in the treated thesis during the growing season. Haifa NIT GG was the fertilizer used for the fertigation. This is a soluble product containing 34,5% of ammonium nitrate. Table 1 shows the fertigation date of each year. The timing for irrigation was determined by leaf water potential measured thanks to a pressure chamber. The thresholds chosen for the control and the treated thesis were 0,8 and 1,2Mpa, respectively.

Fruits and vines sampling.

Grapes have been sampled, and berries chemicals parameters were examined from July to the harvest date. Twenty-one amino acids have been titrated from 3 samples of must per thesis to define their profile among the two treatments at the harvest time. Dormant wood weight, starch, and sugar concentration in both roots and canes tissues have been determined every following winter to detect differences in vigour among treatments. Ten vines per three repetitions in 2020, while five vines per three repetitions in 2021were sampled to measure the vigour indexes. The starch and the sugar content were determined following the methods proposed by HUNTER et al., 1995; and HUNTER J. J. et al., 1995. Yield has been recorded in 2020 weighing the whole production of three consecutive vines per three repetitions in each plot.

Berry chemical analysis

Amino acid concentration has been determined by HPLC-FLD (High-Performance Liquid Chromatography with Fluorescence Detection) exploiting OPA (Orto–phathaldehyde) as a derivatization agent. Chemical analysis of berries juice and starch determination followed official methodology the harvest day.

Winemaking

Grapes from the two treatments have been harvested separately. In 2019 and 2020 the winery turned grapes from each plot into Prosecco PDO sparkling wine. An independent panel taste evaluated the two kinds of Prosecco PDO to assess colour, flavour, aroma, and overall opinion between control and treated wine following the "Union Internationale Des Oenologues" wine sensorial analysis.

Statistical analysis…

Rstudio (version 1.2.1335 © 2009-2019 RStudio) is the software used for all statistical analysis and charts printing. The experimental design chosen was a completely randomized design, split into two treatments: control and nitrogen thesis. The statistical analysis was based on a multifactorial analysis to compare FAA, APA, berries chemicals at harvest, and vigour indexes between thesis and years. Both treatment and year were assessed as fixed effects. Principal component analysis (PCA) detects FAA variation according to the sources of variation.

Table 1 Date of fertigation in each year and dose of nitrogen per vine, total nitrogen supplied per vine and total dose of nitrogen per hectare.

Year	Date	N/Vine (g)	Date	N/Vine (g)	Date	N/Vine (g)	Tot/Vine (g)	Tot/ha Kg/ha
2018	12-Jun	4.33	5-Jul	4.33	17-Jul	4.33	13	38.5
2019	13-Jun	4.33	11-Jul	4.33	31-Jul	4.33	13	38,5
2020	4-Jun	4.33	27-Jun	4.33	31-Jul	4.33	13	38.5

Results

The main climate records from 2018 to 2020 are presented in figure 1. During the vintages 2020 and 2018, the mean temperatures are comparable with the historical trend of the last 20 years, whereas 2019 was a warmer year since June. All three vintage are a bit warmer in August and September than the historical trend. The summer rainfalls were similar to the historical records, except in June

2020 and May 2019 when it rained double. April 2018, April and May 2020, and September 2019 were the most drought months.

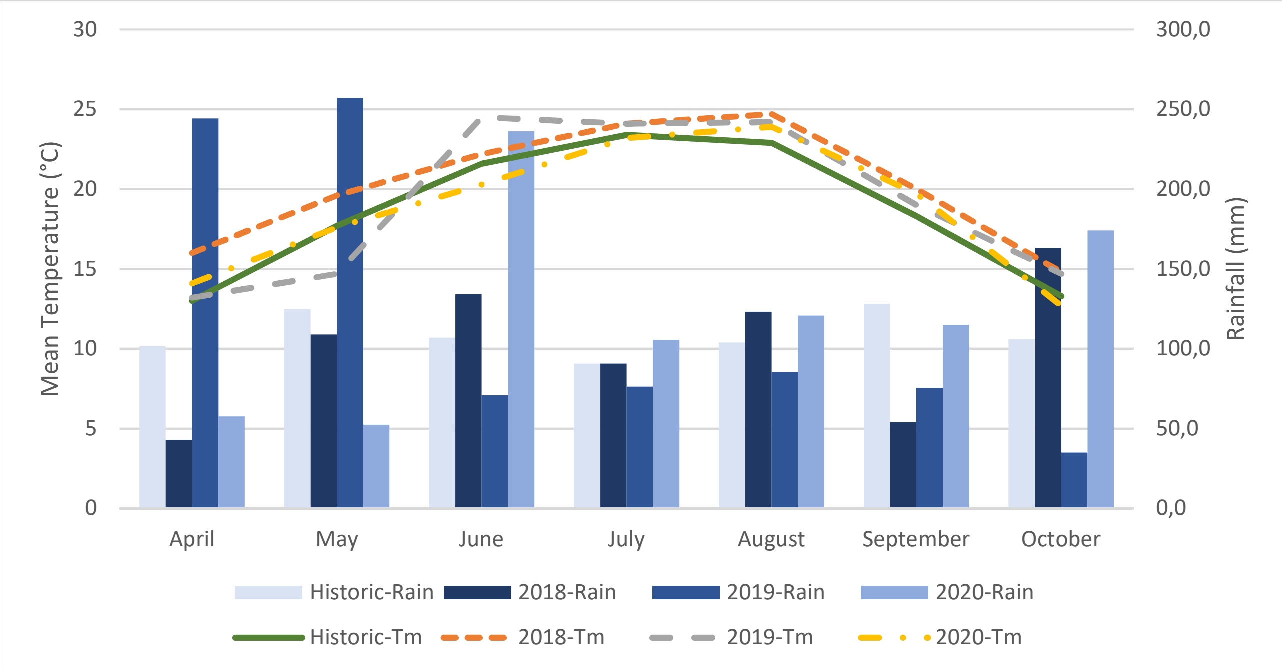


Figure 1. The chart resumes the climate records during the grapevine growing season in the three years compared to the historical trend from 1994 to 2019. Blue columns represent the total monthly rainfall, while lines are the daily average temperature.

The Grapes production has been weighted only in the 2020 vintage and the production is shown in table 2. The timing and the way of nitrogen giving did not affect the total yield in 2020 as verified with the factorial analysis.

Table 2. The grapes yield is expressed in Kg of grapes produced per sample of 3 vines and per each vine..

Thesis	N° Vines	Mean (Kg)	SD	Yield/Vine (Kg)
Nitrogen	3	22.2	3.64	7.4
Control	3	22.08	2.55	7.36

Dormant wood weight, starch and sugar content both in canes and roots are reported in the tables 3,4,5. The above-mentioned parameters showed significant differences between years, but not among the treatments, the outputs of the multifactorial analysis are reassumed in table 6.

Table 3. Mean dormant wood weight, standard deviation and dormant wood weight per vine.

Vintage	Vines	Thesis	Mean (Kg)	SD	Wood/Vine (Kg)
2019	10	Nitrogen	13.3	1.1	1.3
2019	10	Control	14.8	0.4	1.5
2020	5	Nitrogen	7.8	1.4	1.6
2020	5	Control	7.2	1.4	1.4

Table 4. Starch and sugars in the vine's roots are expressed in mg per g of dry matter, SD means standard deviation..

Vintage	Thesis	N° Samples	Starch mean (mg/g dm)	Starch SD	Sugars mean (mg/g dm)	Sugars SD
2018	Nitrogen	3	248.79	10.56	29.76	0.88
2018	Control	3	215.70	37.96	35.73	3.20
2019	Nitrogen	3	227.90	7.52	21.52	5.03
2019	Control	3	192.84	50.01	39.97	23.94
2020	Nitrogen	3	190.33	19.73	16.18	2.76
2020	Control	3	205.01	15.64	16.17	0.20

Table 5. Starch and sugars in vine shoots are expressed in mg per g of dry matter, SD means standard deviation.

Vintage	Thesis	N° Samples	Starch mean (mg/g dm)	Starch SD	Sugars mean (mg/g dm)	Sugars SD
2018	Nitrogen	3	141.92	7.89	126.28	18.21
2018	Control	3	130.40	15.65	120.72	9.50
2020	Nitrogen	3	106.36	8.42	75.45	5.39
2020	Control	3	120.61	7.89	72.85	11.88

*Table 6. Results of the multifactorial analysis on dormant wood weight, starch in roots and shoots, and sugars in roots and shoots. "***" means a p-value< 0.001, "**" means a p-value<0.01, "*"means a p-value<0.05, while "n.s." means not significance.*

Variable	Dormant Wood Weight	Starch Roots	Sugars Roots	Starch Shoots	Sugars Shoots
Vintage	***	n.s.	**	**	**

Treatment	n.s.	n.s.	n.s.	n.s.	n.s.
Interaction	n.s.	n.s.	*	**	**

Yeast assimilable nitrogen (YAN) means the total nitrogen must expressed in ammonium ion (NH4+) concentration. Most organic compounds containing nitrogen are FAA and proteins, whereas mineral compounds are, for example, ions such as NH4+, nitrate (NO3-), and nitrite (NO2-) (Keller 2010; Carlos et al. 2007). Therefore, YAN was always higher in the nitrogen thesis as reported in figure 2 and it differed also in 2019 from the other vintages.

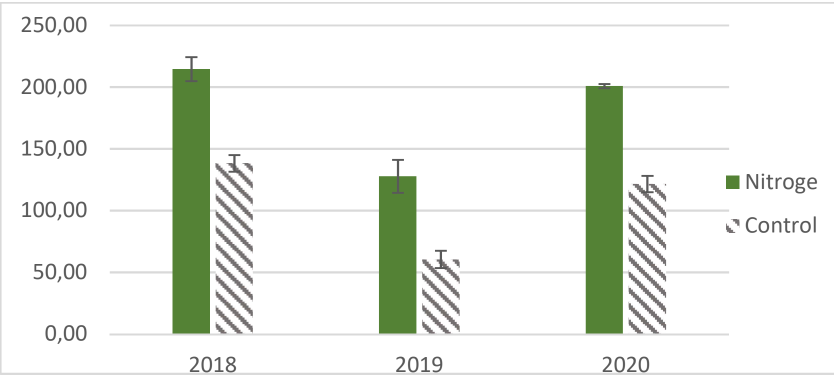


Figure 2. YAN concentration in must at the harvest time. Data are expressed in mg/L of Ammonium nitrate.

The grapes' chemical parameters at harvest time changed over the years and between treatments as proved by the multifactorial analysis which outputs are reassumed in table 7. MA concentration (HMH), the sum of MA and TA concentration (HTH+HMH), the sugars concentration (°BRIX), the sum of all the organic acids (ATT), and pH were significantly different between thesis. TA concentration (HTH), HMH, HTH+HMH, and TTA were significantly different also between vintages. The interaction between treatment effect and vintage was never verified as significant.

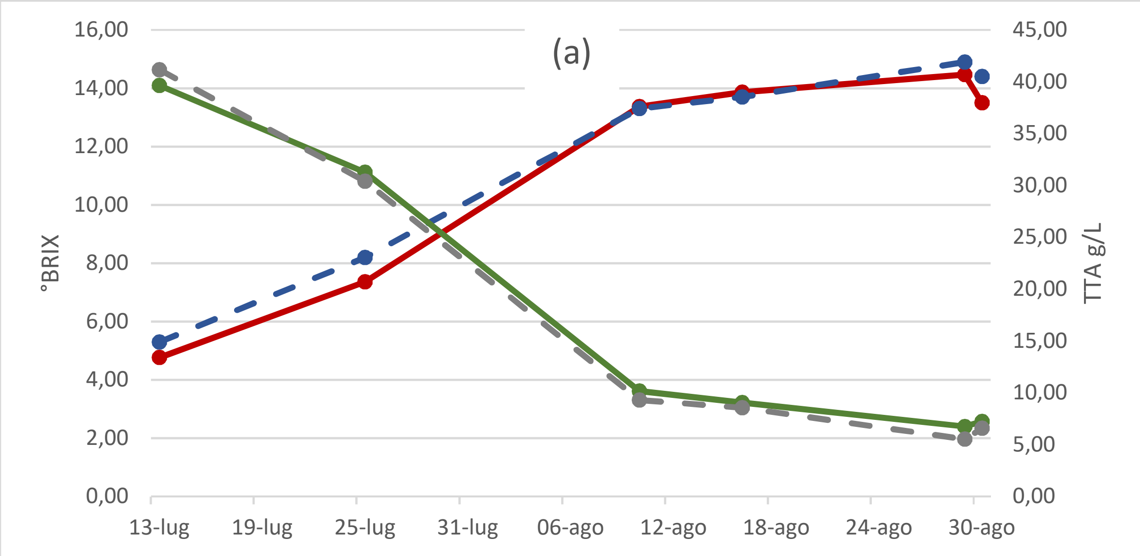
The nitrogen thesis showed every year a higher acidity than the control thesis. pH was lower in the treated than in the control samples. ATT and pH show much variability also among years. The °BRIX (sugar concentration in the berry's juice) showed a weak divergence among the two treatments, which is relatively higher in the control thesis. °BRIX did not vary among years. Measurements are resumed in table 8.

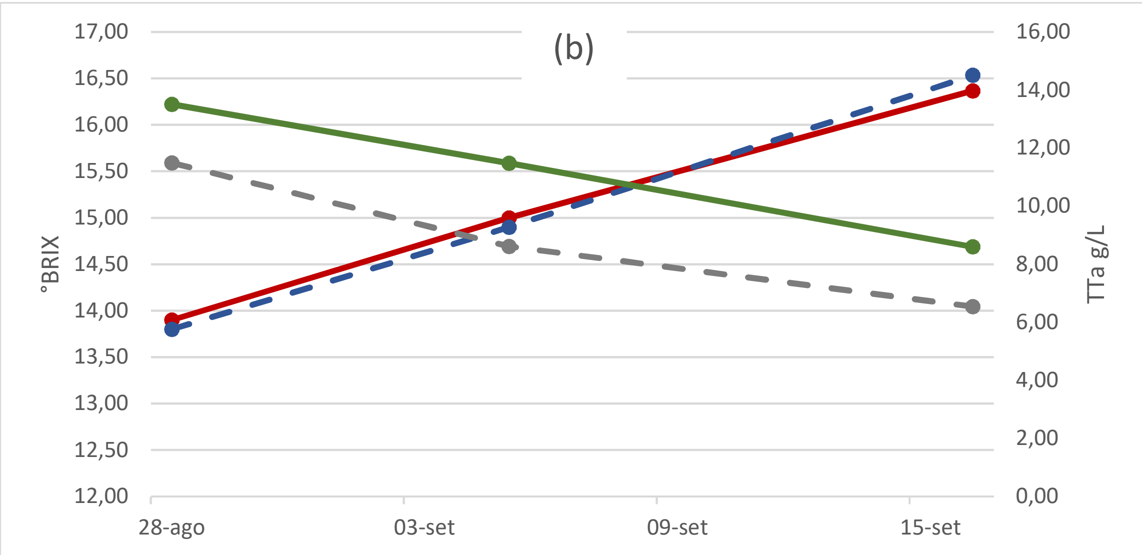
The charts in figure 3 display the °BRIX and TAA evolution from the ripening curve during the vintages. It is clear that acidity falling stopped at a higher value in the nitrogen thesis than in the control one. Regarding °BRIX, it was lower in the nitrogen thesis than the control one since the first sampling in 2018 and 2020.

*Table 7 Results of the multifactorial analysis of chemicals concentration in musts at the harvest time. "***" means a p-value< 0.001, "**" means a p-value<0.01, "*" means a p-value<0.05, while "n.s." means not significance.*

Variable	HTH	HMH	HTH+HMH	BRIX	TTA	pH
Treatment	n.s	***	**	**	***	***

Vintage	*	**	*	***	***	n.s
Interaction	n.s	n.s	n.s	n.s	n.s	n.s





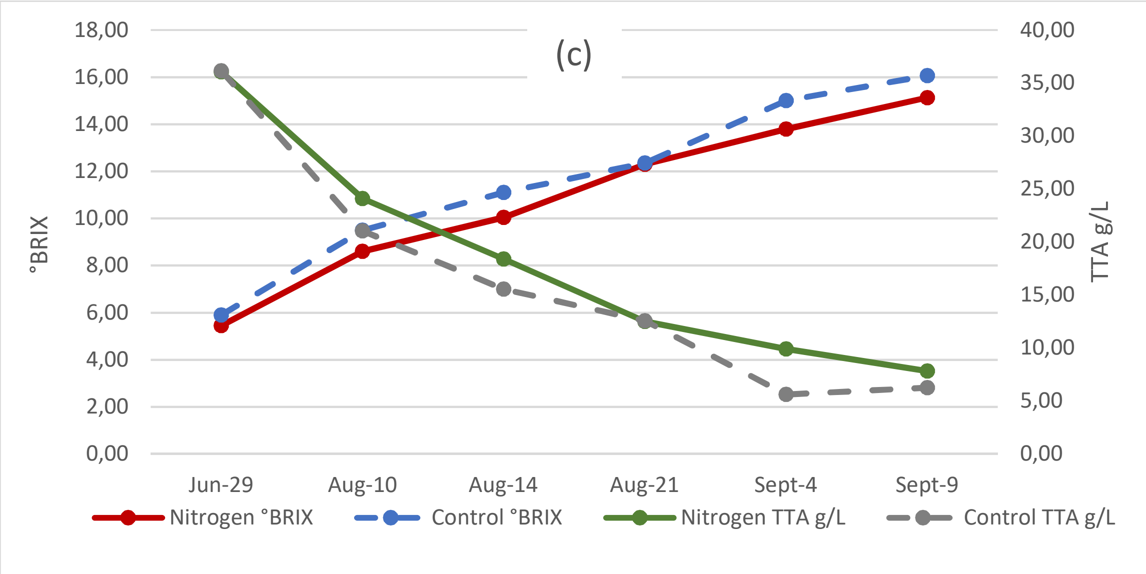


Figure 3. The three charts represent the ripening curve of grapes during the three vintages: 2018 chart (a), 2019 chart (b), and 2020 chart (c). Evolution of °BRIX (left axis) and TTA (right axis) in berries juice from the first available sample to the harvest date.

Table 8. Tartaric acid, malic acid, the sum of tartaric and malic acids, °BRIX, total titratable acidity, and pH in must at the harvest time.

Vintage	Thesis	HTH g/L	HMH g/L	HTH+HMH g/L	°BRIX	TTA g/L	pH
2018	Nitrogen	5.09	2.66	7.75	14.47	6.76	3.22
2018	Control	5.56	1.84	7.40	14.90	5.53	3.30
2019	Nitrogen	6.23	3.42	9.65	16.37	8.60	3.17
2019	Control	5.64	2.20	7.84	16.53	6.54	3.31
2020	Nitrogen	6.70	3.65	10.34	15.13	7.82	3.23
2020	Control	6.08	2.41	8.49	16.07	6.26	3.33

Table 10 resumes the most abundant FAA concentration measured in the must thank the HPLC analysis. The same table reports the results of the multifactorial analysis carried out for each FAA between the two thesis and the three vintages. Arginine, glutamine, aminobutyric acid, alanine, and threonine are the most abundant FAA in the must. The chart in figure 4 illustrates the FAA concentration grouped per treatment. All FAA increased thanks to nitrogen fertilization apart from aminobutyric acid, ethanolamine and glycine. Moreover, the vintage effect was significant towards all FAA except for histidine and threonine. Aminobutyric acid, aspartic acid, arginine, citrulline, glutamine, and lysine showed significant year and treatment interaction. The concentration of the FAA did not increase equally among each amino acid. Citrulline, glutamine, arginine, and tyrosine rose by more than 25% in concentration in the treated thesis.

The first and the second components of the PCA explained the 40.35% and 31.35% (71.7% together) of the total variance respectively. Figure 5 plots each FAA score according to the first and the second dimension. The score distribution shows clearly the effect due to the treatment, while it is not so clear the vintage effect in the same way.

The panel evaluated the Prosecco wine made with the grapes coming from the fertigated parcel better for smell and taste sensation than the control wine. Tasters assessed that the wine was more pleasant thanks to the higher acid content, and more aromatic than the control wine.

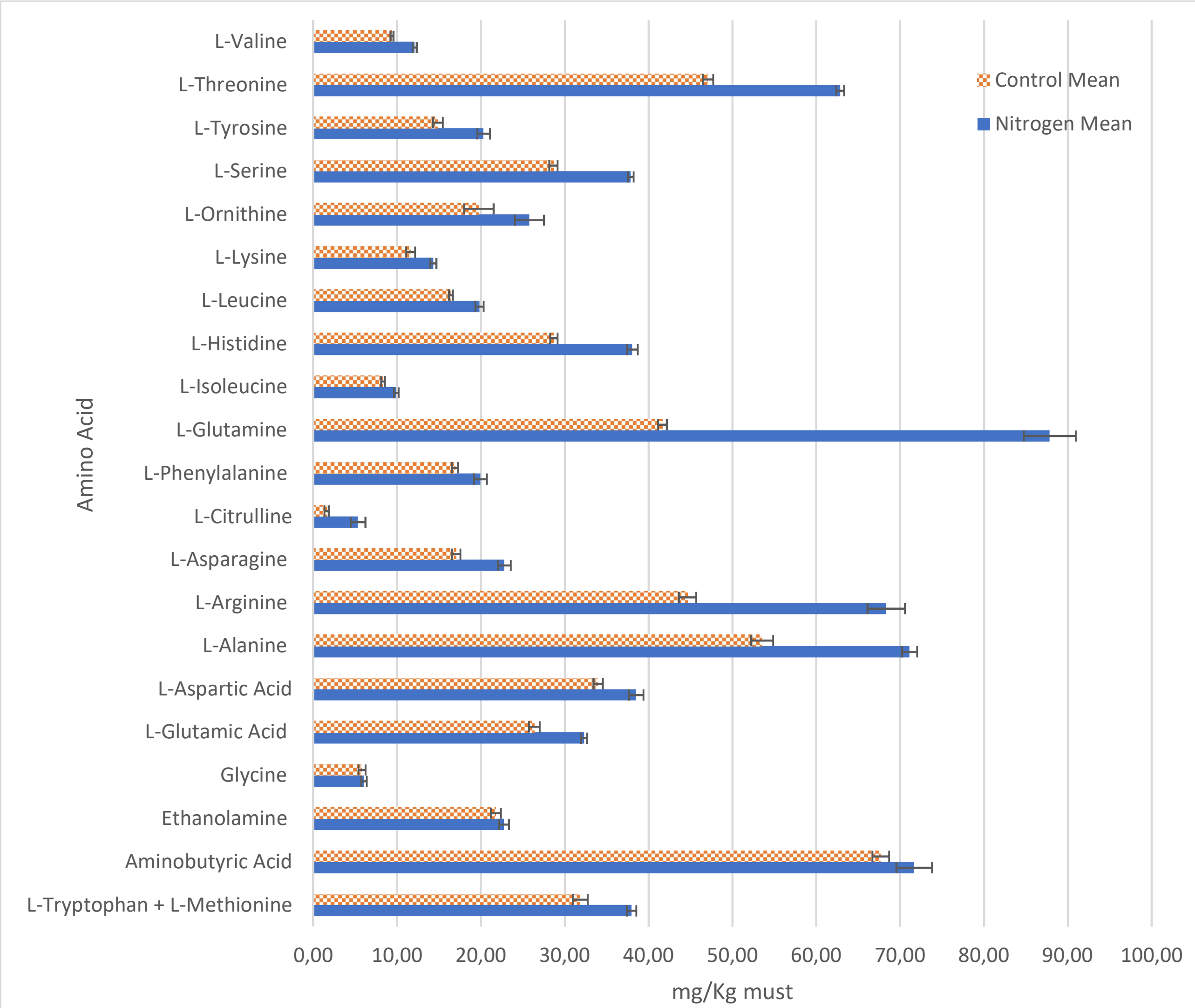


Figure 4. The histogram resumes the average concentration of the FAA in must, at the harvest time grouped per treatment. The black bars represent the mean standard error. L-Arginine concentrtions are reduced by ten times.

*Table 10. FAA mean concentration (three samples) in mg/Kg of must at harvest time. The measures are divided in treatments each year. Multifactorial analysis represents the ANOVA results from the comparison of treatment, vintage and their interaction effect on FAA concentration. . "***" means a p-value< 0.001, "**" means a p-value<0.01, "*" means a p-value<0.05, while "n.s." means not significance.*

Vintage	2018				2019				2020						
Treatment	Nitrogen		Control		Nitrogen		Control		Nitrogen		Control		Multifactorial analysis		
Amino acid	Mean	SD (n=3)	Mean	SD (n=3)	Mean	SD (n=3)	Mean	SD (n=3)	Mean	SD (n=3)	Mean	SD (n=3)	Treatment	Vintage	T x V
L-Tryptophan + L-Methionine	39.70	1.82	33.03	1.01	33.90	2.69	24.70	2.88	40.33	7.46	37.80	10.96	*	*	NS
Aminobutyric Acid	75.27	10.12	69.10	8.68	90.83	1.50	73.23	10.37	48.93	2.25	60.73	3.69	NS	***	*
Ethanolamine	16.17	1.01	16.50	0.95	24.03	0.60	20.60	2.79	28.10	0.72	28.27	0.85	NS	***	NS
Glycine	4.23	0.84	3.23	0.45	3.83	0.38	3.33	0.78	10.03	0.25	10.87	1.42	NS	***	NS
L-Glutamic Acid	32.50	2.27	25.97	3.40	29.20	2.17	20.70	2.62	35.23	1.45	32.47	3.07	***	***	NS
L-Aspartic Acid	31.83	2.00	28.30	3.56	47.43	5.99	36.37	2.51	36.30	1.39	37.27	2.52	*	***	*
L-Alanine	80.27	5.70	66.23	9.65	66.50	6.17	41.77	2.16	66.63	1.39	52.63	1.60	***	***	NS
L-Arginine	488.17	19.64	380.43	18.58	625.00	33.51	392.67	40.53	937.23	31.74	566.50	5.06	***	***	***
L-Asparagine	20.50	3.66	17.13	0.12	31.23	0.70	20.73	2.11	16.70	0.26	13.27	5.95	**	***	NS
L-Citrulline	0.10	0.00	0.10	0.00	0.10	0.00	0.10	0.00	15.83	0.74	4.57	1.59	***	***	***
L-Phenylalanine	21.83	4.92	18.17	1.30	26.07	3.00	19.57	1.19	11.93	0.29	13.00	1.87	*	***	NS
L-Glutamine	62.00	12.28	45.90	5.23	80.23	12.29	41.77	2.02	121.33	2.95	37.27	1.86	***	***	***
L-Isoleucine	10.83	2.44	10.63	0.81	11.77	1.50	8.20	0.66	7.13	0.35	6.13	1.11	*	***	NS
L-Histidine	33.37	6.77	26.70	4.23	43.23	4.03	26.70	2.33	37.60	1.28	32.70	2.63	***	NS	NS
L-Leucine	16.90	2.34	15.53	1.00	25.13	3.45	17.97	2.46	17.47	0.23	15.73	2.27	**	**	NS
L-Lysine	12.13	0.61	7.60	0.10	12.13	0.50	9.27	0.71	18.70	0.26	17.97	1.22	***	***	**

L-Ornithine	14.77	0.93	8.67	0.55	16.00	0.98	9.57	0
L-Serine	39.17	1.18	30.93	3.79	34.67	2.91	23.77	1
L-Tyrosine	28.80	3.82	21.47	3.03	16.97	1.86	11.30	1
L-Threonine	64.03	2.42	48.53	6.09	60.40	7.01	41.93	4
L-Valine	13.57	2.40	10.90	1.73	12.70	1.61	8.27	0

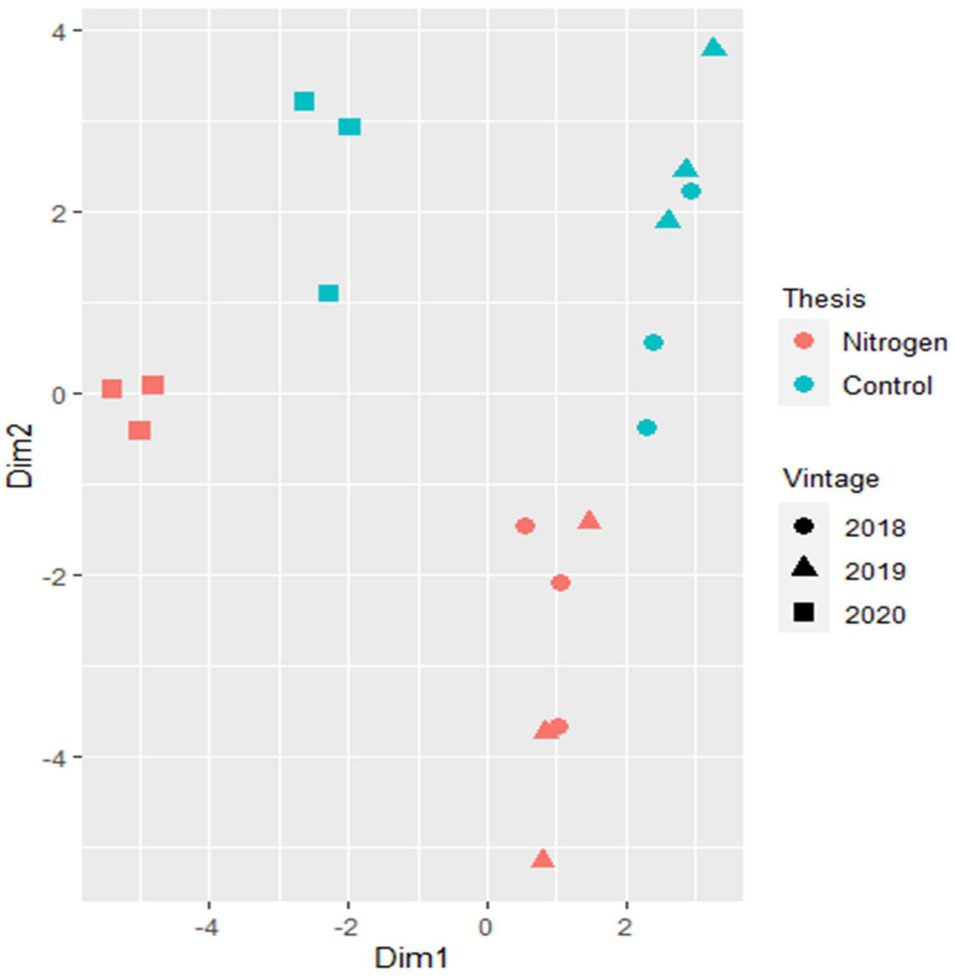


Figure 5. The scores of the FAA from the PCA. Three repetitions were sampled from 2 treatments in three years for a total of 18 points plotted in the chart. Each point has been grouped by treatment, colour, and vintage, shape.

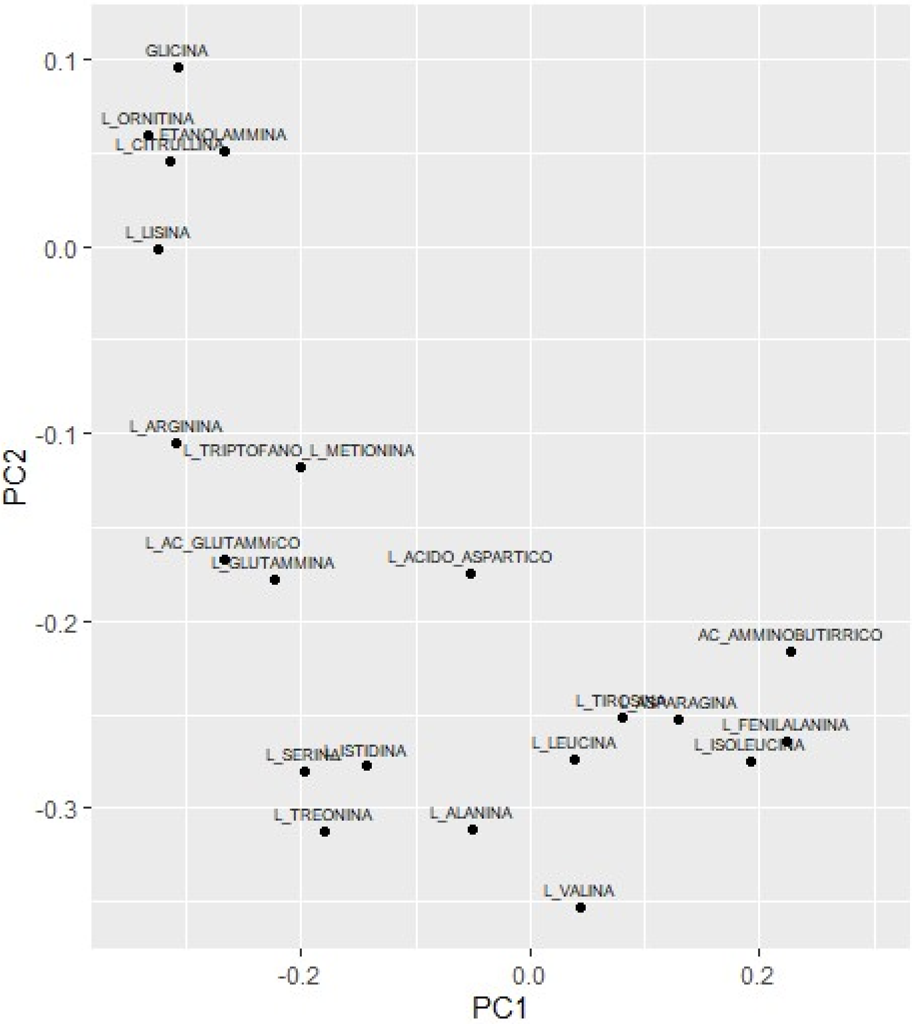
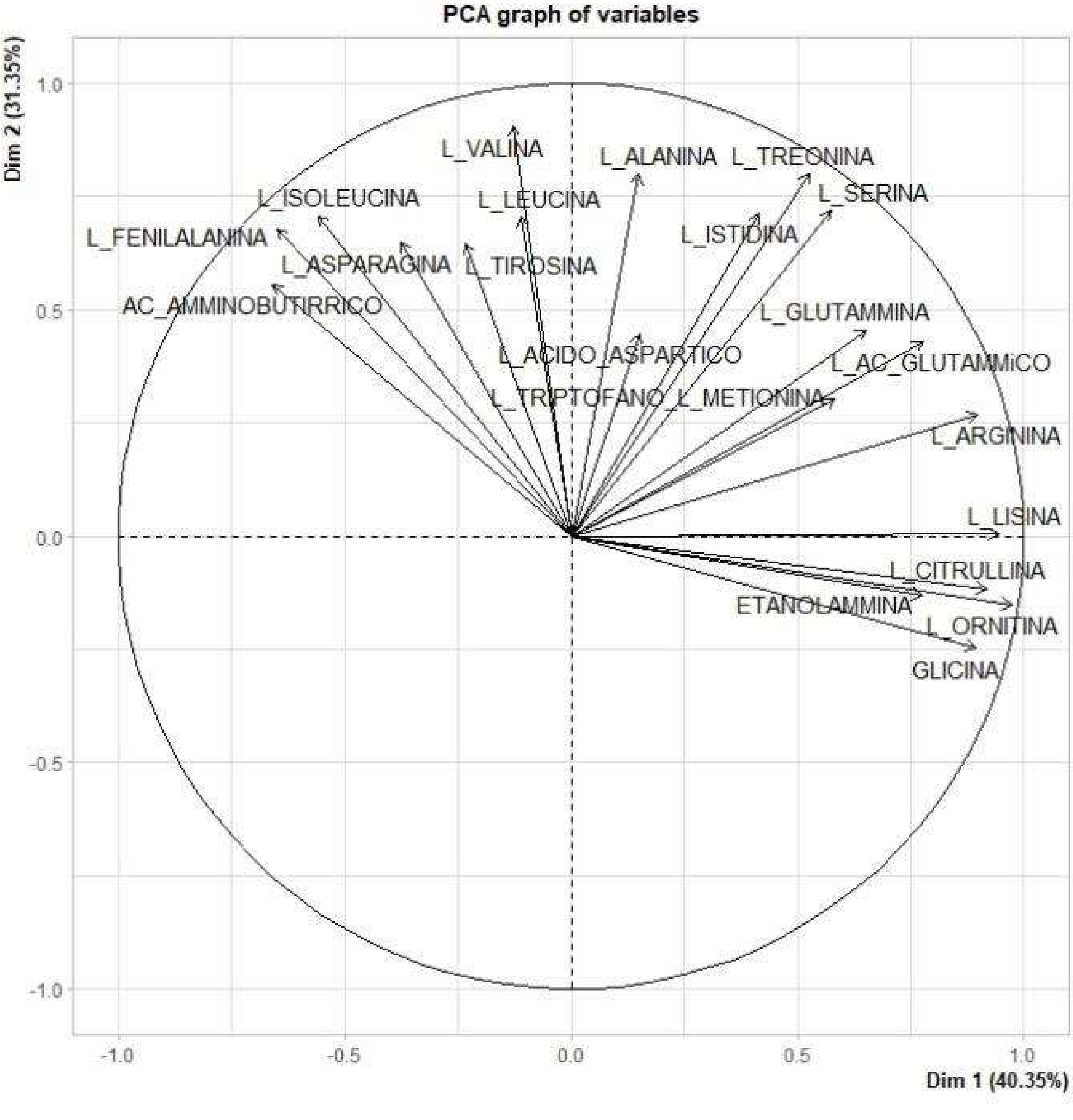


Figure 1PCA FAA loadings


PCA graph of variables
Dim 2 (31.35%)
Dim 1 (40.35%)
1.0
0.5
0.0
-0.5
-1.0
-1.0
-0.5
0.0
0.5
1.0
L_VALINA
L_ALANINA
L_TREONINA
L_SERINA
L_ISOLEUCINA
L_LEUCINA
L_FENILALANINA
L_ISTIDINA
L_ASPARAGINA
L_TIROSINA
AC_AMMINOBUTIRRICO
L_GLUTAMMINA
L_ACIDO_ASPARTICO
L_AC_GLUTAMMiCO
L_TRIPTOFANO
L_METIONINA
L_ARGININA
L_LISINA
L_CITRULLINA
ETANOLAMMINA
L_ORNITINA
GLICINA

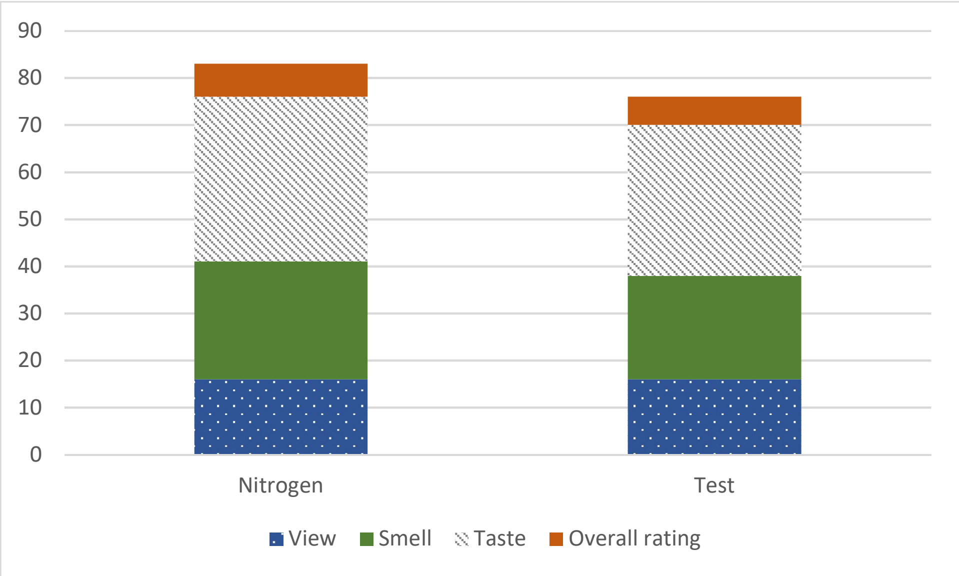


Figure 6. The histogram sum of the scores of each wine rating from the panels test. Sensorial anlysis was divided into three assessment View, Smell, Taste and the final Overall rating.

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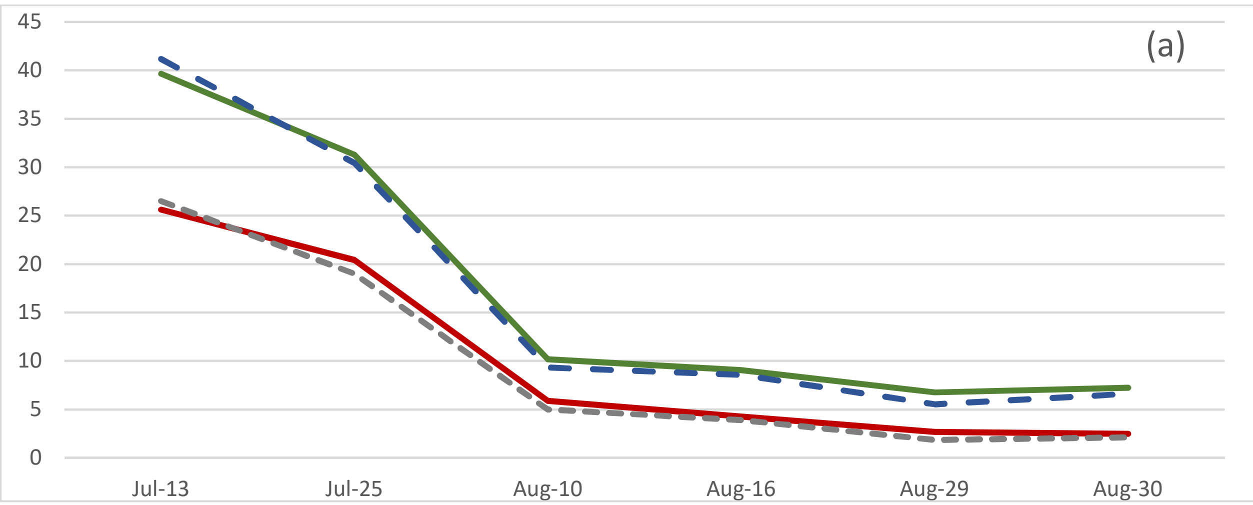
Discussions

From the climate comparison of the three years, 2019 was the hottest vintage. Moreover, in 2019 it rained more in April and in May. 2018 and 2020 vintages are comparable with the historical trend, but 2020 was very rainy in June. Each year's climate condition could play a crucial role in boosting the vintage effect in the statistical analysis.

The dormant wood weight, starch and sugar content in roots and shoots has been measured to assess the vine's vigour. The vigour assessment proved nitrogen fertigation did not stimulate the vines' vegetative growth. Starch's stocks and dormant wood weight excluded any nitrogen accumulation effect in the soil. The plant vigour of the fertigated thesis followed the same trend of the not-fertigated one without increasing year by year and avoiding issues related to luxury vegetation in grapevines. In addition, to support this hypothesis, nitrogen fertigation did not affect yield in 2020, which was the last year of the experimental trial. Dense canopies are harmful to grapevines. Dense canopies could involve adverse effects on berry juice and the plant's health (Tesic, Keller, and Hutton 2007). It has been also proved that vigour vines are more susceptible to phytophagous insects and fungal diseases (Mundy 2008).

The analysis of the chemical parameters proved that thanks to the nitrogen fertigation the acidity of the berries juice increased whereas reducing the sugar concentration. In particular, HMH and TTA were higher in the nitrogen thesis, while HTH was similar in both the parcels. In all the vintages, HMH and TTA were higher in the nitrogen plot since the first samplings. Moreover, looking at the chart in figure 6, the acidity falling is less strong in the nitrogen thesis than in the control one for all the vintages.



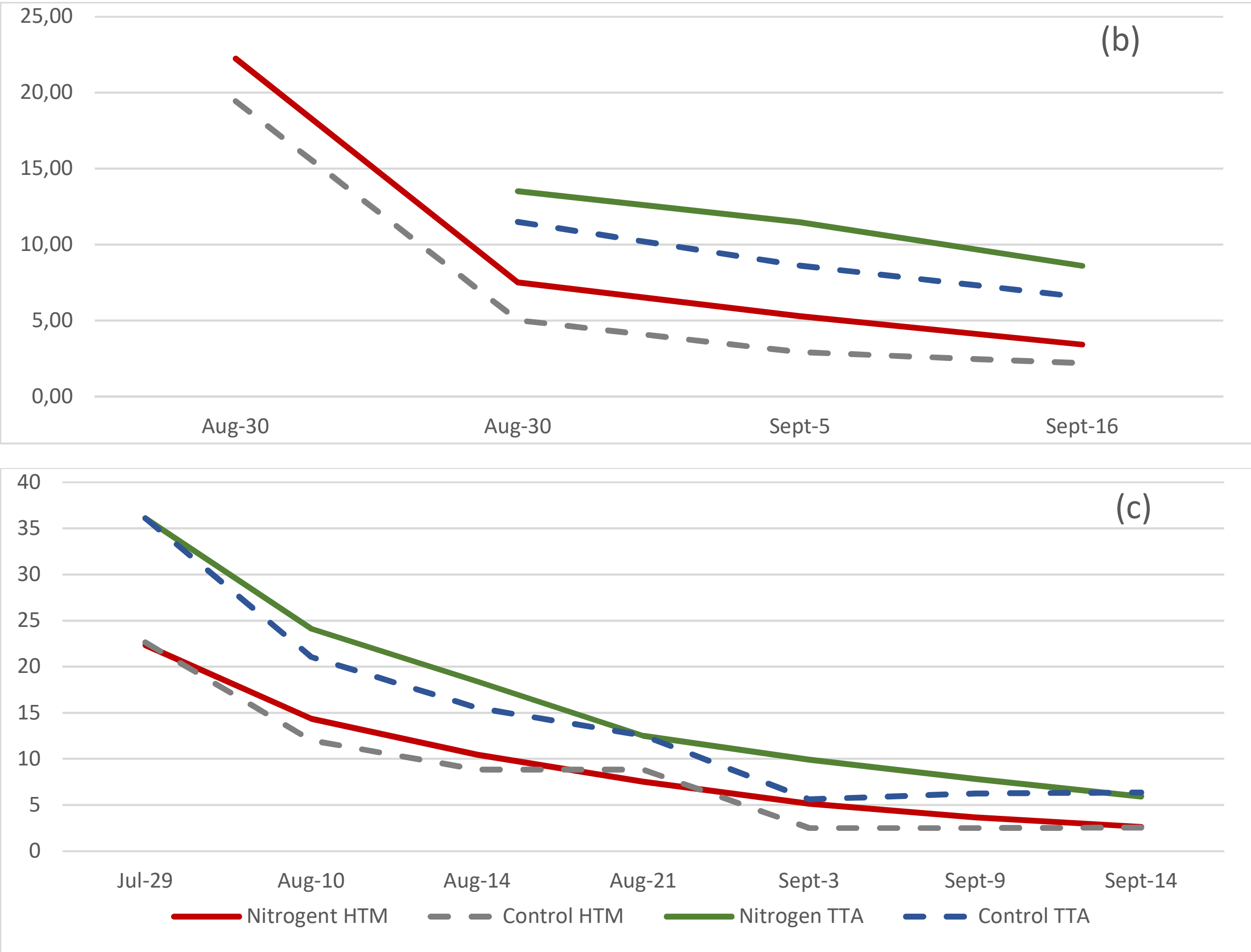


Figure 6. The three charts show the acidity falling during the grapevine growing season of each year: 2018 chart (a), 2019 chart (b), and 2020 chart (c). Malic acid concentration and total titratable acidity are expressed in g/L.

YAN depend on many factors, among which nitrogen fertilization (Hannam et al. 2013). YAN is an essential macronutrient for yeast during alcoholic fermentation, and many nitrogen compounds are precursors of phenolics and many other aromatics compounds (Carlos et al. 2007; Keller 2010; Vilanova et al. 2007; Hannam et al. 2013).

Usually, the musts contain more than thirty amino acids, but the seven most abundant ones represent more than 78% of the overall concentration of amino acids, as verified by Carlos et al. 2007. Arginine is the most abundant FAA found in berry juice. Likewise, many other studies proved this. Arginine synthesis is a common way to store nitrogen in the berries juice (Xia and Cheng 2004; Cook and Kliewer 1971), and it is also the major source of nitrogen for the yeasts during alcoholic fermentation (Bell and Henschke 2005). Arginine is the predominant FAA in both the thesis, and it also increases by 53% thanks to fertigation in the three vintages mean. The concentration of 18 out of 21 of the analysed amino acids increased in the must coming from fertigated grapes, as verified by Schreiner et al., 2014.

All the monitored FAA, apart from histidine and threonine, change in concentration across vintages. Climate conditions, sun exposure, farming practice and many other unpredictable patterns could have affected the FAA concentration over years.

The main differences between the Prosecco wine coming from the two parcels are almost due to the acidity and the FAA composition. The wine acidity changes the wine's taste, while the FAA composition affects the aromas in the wine. For example, it has been proved the crucial role of phenylalanine as a chemical precursor of phenolic compounds, the aromatic molecules responsible for wine aromas (Keller 2010; Carlos et al. 2007; Styger, Prior, and Bauer 2011; Parker et al. 2018). In addition, other studies verify the relation between the concentration of threonine, aspartic acid, and phenylalanine as the FAA is much related to the wine aromas (Hernández-Orte, Cacho, and Ferreira 2002; Roda et al. 2019; Styger, Prior, and Bauer 2011). Generally, higher concentrations of amino acids in the berries often result in higher concentrations of fruity and floral esters and thiols and in lower concentrations of higher alcohols (Keller 2010).

Conclusions

The present research study aimed to improve the oenological features of grapes to produce high quality white sparkling wine as Prosecco PDO. 13 g/vine of nitrogen has been provided thanks to a soluble mineral fertilizer melted in the irrigation water. Three fertigations supplied 38,5Kg/ha of nitrogen from vines flowering to grapes veraison. After flowering, the control plot received a granular mineral fertilizer spread in a unique passage. Although the treated parcel received a higher dose of nitrogen, Dormant wood and nutrients stored in both roots and shoots did not increase in the treatment plot compared to the control one. Therefore, neither the grape's yield has been affected by the fertigation. On the other hand, the three fertigation events increase the acidity in the berries juice and FAA in must. Finally, more acidity and FAA involved more pleasant, aromatic and fresh wine, respecting the wine obtained from the control parcel.

Acknowledgements

This study could take part thanks only to the contribution of winery c/o Vazzola and Haifa. The trial took place c/o Vazzola vineyard, and the grapes were processed in the same winery owned by same company as Vazzola. Furthermore, Haifa provided soluble fertiliser each year, and they also installed a system to mix the irrigation water with the fertiliser.