

CHARACTERIZATION OF NEBBIOLO WINES IN PROVINCE OF TURIN AND IN LOWER AOSTA VALLEY

Vittoria Brugnago

Dissertation to obtain a Master's Degree in
Master Degree in Engenharia de Viticultura e Enologia

SUPERVISOR: Prof. Vincenzo Gerbi

SUPERVISOR: Prof. Jorge Manuel Rodrigues Ricardo da Silva

PRESIDENT :

PhD Sofia Cristina Gomes Catarino, Assistant Professor at Instituto Superior de Agronomia, Universidade de Lisboa

MEMBERS :

PhD Vincenzo Gerbi, Full Professor at Università degli Studi di Torino

PhD Manuel José de Carvalho Pimenta Malfeito Ferreira, Assistant Professor with Habilitation at Instituto Superior de Agronomia, Universidade de Lisboa

2019

To Filippo,

that always shows the best part of me.

Acknowledgments

Here we are again, after exactly two years, thinking of those who in my life represented a moment of joy, pain, smiles, salvation anchors, shoulders to cry on and above all laugh... laugh so much.

First of all I thank Gabriella and Marco, splendid parents who supported me, handle me, made magnificent travels and gave birth to the mad woman I am now. I warmly thank the nūni Ago, who has raised me since I was a "microbino", by spoiling me and giving me all the love of the world. To my brother Alessandro, who wanted absolutely a sister and if I were born a boy, he would surely asked to change me in the market; brother who taught me to be with the grown-ups, but above all with the "males". I thank my nephew Filippo, who has been filling my heart with unconditional love for almost two years. I thank my aunt Laura, who with her laughter and aperitifs is a confidante, friend and comfort. To Nino, who makes my mother happy, me happy and brought home the Sicily that was missing.

Let us now turn to the "piezz e core". I thank my best friend Ilaria, an anchor who has been present in the moments of celebration and pain for the past 14 years. SHE IS ALWAYS PRESENT. Laura, known at the university, who with her grit and her "monatudine" made me a little Venetian and filled my heart with emotions. I would not have survived without her. I say thanks to my clubbing friends, Sara, Roberta and il Morbido, always gassed and ready to go to move the hips, but also to welcome my sad drunks at 4 am. To Fabione, who always to take me photos of unimaginable ugliness, but we know that it's a way to show us affection. To Rudi, who accompanies me in my career and life, as a friend and winemaker to the sound of "birrette" and to "us winemakers ...". To Bardonecchia and my second mountain family, all the friends who are always willing to drink a genepy together.

I thank you for having chosen to continue studies, doing the specialist degree. Without this I would not have known so many pieces of my heart. Thanks to Michele, Andrea, Federico for the vinitaly 2019: journeys of hope, big wine talks, companions of idiocy and wine ... so much wine.

And now Lisbon. Thank you for all the emotions you give me, for making me drink, smile, laugh, admire your beauty, your sunsets, Santos, your saudade (which still strikes me now), built memories and a new family made up of many people, suffer the cold, the difficulties and made cry ... so much. Thanks to Mariana, a beautiful Portuguese soul that I will always carry with me and on whom I can always count, she who calls me "Honey b". Thanks to Anna and Beatrice, always with me both from near and far, traveling companions who with "there is stink of the Azores" knew how to start travels by car. Perhaps there are no people who can better understand every moment I have lived with them, without them both in Lisbon and back home.

Thank you. Thanks to Damiano, Adelaide, Andrea, Alessandro, Filippo, Gavino, Cico, Peppe, Marta, Ruben who accompanied me in this second year in Lisbon; you made me feel at home. To "Stormi" that in my ears has been reproduced so many times on that 760 and above the bridge of April 25, the first time I returned to Lisbon. To you ... you know how to make me rejoice like nobody ever, but at the same time you have the ability to make you hate in 1 second. You supported me, helped me, destroyed and reassembled, made to shed so many tears. To you with whom it all seemed like a dream, but also a nightmare. To you who would like you here, but I wouldn't want you here. To you ... I can't even write your name.

To all of you, I say thanks and on this special day, you will all be with me, both physically and mentally. I love you and my heart embraces you.

Até já

Vittoria

Abstract

This work was born as a consequence of an ALCOTRA project, called "Alpine Vineyard Road". The wines examined are 22 in total, made up of at least 85% of Nebbiolo, produced in the province of Turin and in the lower Aosta Valley, between 2018 and 2015. The focus of this thesis is on the winemaking methods adopted, the phenolic composition and its effects on the color of the wines. In this phase of the work, the samples were compared with other research carried out in more well-known areas such as Langhe and Roero and with Nebbiolo of Carema, Barbaresco, Donnas. By fractionation of the color of wines at pH 0, we obtain the percentages of anthocyanins monomers, the pigments linked to the tannins and stable and pigments that are decolourable with the addition of sulfur dioxide. The values of the colour intensities of the samples are below the average of the values recorded compared to the results of the other studies. As for the colour tone, which is higher, the more a wine will tend to orange tones, at least half have results with high values and therefore tending towards orange. The last analysis carried out is the measurement of polymeric pigments of wines that uses a protein precipitation, precisely bovine albumin serum (BSA), combined with bleaching with bisulfite. Polymer pigments are made up of anthocyanins linked to tannins, these can be differentiated into two groups: those that do not precipitate with proteins, short polymeric pigments (SPP) and those that precipitate with proteins, given their size, long polymeric pigments (LPP). This work contributes to the qualitative and quantitative knowledge of the wines of the territories examined. Finally, the aim is to have a wider knowledge of an area, which has previously been ignored, but for some years now, has returned to attention for a re-evaluation of the territory.

Key words: Nebbiolo, colour, Turin, Aosta Valley, phenolic composition

Resumo

O presente trabalho surgiu na sequência do projeto ALCOTRA, denominado "Strada dei vigneti Alpini". Foram examinados 22 vinhos no total, constituídos por pelo menos 85% de Nebbiolo, produzido na província de Turim e no vale de Aosta, entre 2015 e 2018. Esta tese pretende enquadrar os métodos de vinificação adoptados, assim como a composição fenólica e os seus efeitos na cor dos vinhos. Nesta fase do trabalho, as amostras foram comparadas com outras pesquisas realizadas em regiões mais conhecidas, como Langhe e Roero e com Nebbioli di Carema, Barbaresco, Donnas. Com o fracionamento da cor dos vinhos a pH 0, obtêm-se 3 frações, que são as antocianinas monoméricas, os pigmentos estáveis ligados aos taninos e por fim os pigmentos que podem ser descorados pela adição de dióxido de enxofre. Os valores de intensidade de cor das amostras estão abaixo da média dos valores registados em relação aos resultados dos outros estudos. Quanto à tonalidade de coloração, quanto maior, mais o vinho tenderá a tons de laranja. Pelo menos metade deles apresentam resultados com valores altos e, portanto, tendem a laranja. A última análise realizada foi a medição de pigmentos poliméricos em vinhos utilizando a precipitação de proteínas, nomeadamente a albumina de soro bovino (BSA), combinada com o branqueamento com bissulfito. Os pigmentos poliméricos são compostos de antocianinas ligadas a taninos, estes podem ser diferenciados em dois grupos: aqueles que não precipitam com proteínas, pigmentos poliméricos curtos (SPP) e aqueles que precipitam com proteínas, dado seu tamanho, pigmentos poliméricos longos (LPP).

Este trabalho contribui para o conhecimento qualitativo e quantitativo dos vinhos dos territórios examinados. Finalmente, o objetivo é ter um conhecimento mais amplo da região, antes ignorada, e que desde há alguns anos, captou o interesse para uma reavaliação do território.

Palavras chave: Nebbiolo, cor, Torino, Valle d'Aosta, composição fenólica

Resumo alargado

Este trabalho, realizado no ano 2018/2019, nasceu graças a um projeto da ALCOTRA, chamado "Alpine Vineyard Road". Os objetivos do projeto são o aprimoramento dos vinhos, paisagens e vinhedos das áreas envolvidas e dos próprios produtores para fins turísticos. Os vinhos examinados são 22 no total, produzidos em 20 empresas privadas e cooperativas sociais localizadas na província de Turim (Canavese, Carema, Pinerolese) e no baixo vale de Aosta. Pelo menos 85% das amostras examinadas eram Nebbiolo pertencentes às vindimas entre 2015 e 2018. Os parâmetros analisados foram: antocianinas monoméricas individuais e totais, a estabilização e sensibilidade da matéria corante, os índices colorimétricos (CIELab) e finalmente ao tamanho dos pigmentos corados. As fichas técnicas mostram uma grande variabilidade nos dias de maceração nas películas, desde mais curtos e inovadores (5 dias) aos tradicionais e longos 90 dias. No que diz respeito à maturação, também neste caso, temos grande variedade: a amostra número 2, por escolha do fabricante, não matura. Em vez disso, a amostra número 14 estagiou 36 meses em madeira de vários tamanhos (barrica, tonel e quartola).

As análises das antocianinas totais e monoméricas individuais, comparadas com trabalhos realizados em Nebbiolo di Carema, Barbaresco, Donnas e Langhe em geral, mostram valores abaixo da média. Fraccionando a cor dos vinhos a pH 0, obtêm-se as frações de antocianinas monoméricas, os pigmentos estáveis ligados aos taninos, e ainda os pigmentos descoloráveis com a adição de dióxido de enxofre. Os resultados das amostras número 5 e 6, ambas de 2017, mostram situações opostas: a número 5 possui uma percentagem de pigmentos sensíveis ao SO₂ de 58% e apenas 9% de pigmentos estáveis não sensíveis. A amostra número 6, por outro lado, apresenta 60% de pigmentos estáveis, envolvendo antocianinas e taninos, e não sensíveis ao dióxido de enxofre.

50% das amostras, em relação aos valores das intensidades de coloração, estão abaixo da média em comparação com os valores obtidos em outros estudos realizados no Nebbiolo. Quanto à tonalidade da coloração, que é mais alta, mais um vinho tende a tons de laranja, 8 das 22 amostras mostram resultados maiores que 1 e, portanto, tendem a laranja. Finalmente, foi realizada uma análise que mede os pigmentos poliméricos dos vinhos utilizando uma precipitação de proteínas, precisamente soro de albumina bovina (BSA), combinada com o branqueamento com bissulfito. Os pigmentos poliméricos são compostos de antocianinas ligadas a taninos, que podem ser diferenciados em dois grupos: aqueles que não precipitam com proteínas, os pigmentos poliméricos curtos (SPP) e aqueles que precipitam com proteínas, devido ao seu tamanho, denominado pigmentos poliméricos longos (LPP). Estes beneficiam a estabilidade da cor do vinho, uma vez que quanto mais SPP e LPP são formados

e formados, mais a cor será fixada. Nos estágios iniciais da vinificação, mais SPPs são formados; em vez disso, a formação de LPP é a mais favorecida durante o envelhecimento. Todo o Nebbiolo da vindima de 2018, depois de recentemente vinificado, tem um teor mais alto de monômeros de antocianinas do que o restante dos vinhos, pelo contrário, o SPP tem as menores porcentagens entre as amostras. Apesar de serem vinhos muito jovens, os valores de LPP parecem superiores aos pigmentos poliméricos curtos. O envelhecimento na madeira, ajuda na polimerização entre antocianinas e taninos; de fato, o valor mais baixo é encontrado na amostra número 2, que não estagiou em madeira.

Em conclusão, este trabalho fornece uma contribuição não definitiva ao conhecimento qualitativo e quantitativo dos vinhos dos territórios examinados. Finalmente, o objetivo é ter um conhecimento mais amplo de uma área, que já havia sido ignorada, mas há alguns anos, voltou a atenção para uma reavaliação do território.

Dos 22 vinhos analisados, a marca da presença da casta Nebbiolo, a sua localização, o manejo da vinha, clima e altitude que afetam profundamente a expressão do vinho são perfeitamente reconhecíveis.

A cor, um ponto fundamental na degustação de um vinho, deve ser protegida, levada em consideração como uma força do produto e mais conhecida pelos produtores. No entanto, os resultados confirmam que o Nebbiolo dessas áreas se expressa de uma maneira potencialmente interessante também do ponto de vista comercial.

Na minha opinião pessoal, os Nebbioli localizados fora das famosas áreas de produção de Barolo e Barbaresco devem receber atenção voz, não apenas pela grande força que a cultivar representa nas áreas estudadas, mas também porque a denominação de origem, são também “compradas”, muitas vezes em maior extensão que a própria qualidade do conteúdo da garrafa.

INDEX

1. INTRODUCTION.....	12
2. LITERATURE REVIEW.....	13
2.1. The history of Nebbiolo.....	13
2.2. The origin of Nebbiolo.....	14
2.3. Landscape and architecture of Nebbiolo.....	16
2.3.1. The abandoning of pergolas and vineyards.....	18
2.4. Phenolic profile and the Nebbiolo oenology.....	19
2.4.1. Phenolic profile of Nebbiolo.....	19
2.4.2. Vinification of Nebbiolo.....	21
2.4.3. The colour of Nebbiolo.....	22
2.4.3.1. Maceration and stabilization of the colour.....	22
2.4.3.2. Colorimetric indexes.....	23
3. AIM OF WORK.....	24
4. MATERIALS AND METHODS.....	25
4.1. Company survey form.....	25
4.2. Chemical analysis.....	27
5. RESULTS AND DISCUSSION.....	28
5.1. Company survey form – company description.....	28
5.1.1. Company, vineyard surface and wine type.....	28
5.1.2. Location and altitude (map).....	30
5.1.3. Training system and pruning system.....	30
5.1.4. Nebbiolo grown in company.....	31
5.1.5. Vineyard management.....	31
5.1.6. Grapes and wine analysis.....	32
5.1.7. Bulk/bottled.....	33
5.2. Company survey form – description of production technique and equipment used.....	34
5.2.1. Nebbiolo blend.....	34
5.2.2. Crushing.....	35
5.2.3. Fermentation – Yeast inoculum.....	35
5.2.4. Fermentation – Tank.....	36
5.2.5. Fermentation – T° Control.....	36
5.2.6. Malolactic fermentation – Tank.....	37
5.2.7. Malolactic fermentation – Inoculum.....	37

5.2.8. Malolactic fermentation – Heat.....	38
5.2.9. Fining and stabilization.....	38
5.2.10. Aging.....	39
5.2.11. Aging container.....	39
5.3. Base analysis.....	41
5.3.1. Residual sugar.....	42
5.3.2. Total acidity.....	42
5.3.3. Volatile acidity.....	42
5.3.4. pH.....	43
5.3.5. Malic acid.....	44
5.3.6. Lactic acid.....	44
5.4. Phenolic and colour analysis.....	45
5.4.1. Total polyphenols.....	45
5.4.2. Total flavonoids.....	46
5.4.3. Total and monomer anthocyanins.....	47
5.4.4. Proanthocyanidins.....	49
5.4.5. Vanillin reactive flavanols.....	50
5.4.6. The colour.....	51
5.4.6.1 Fractionation and wine color indexes at pH0 (%).....	51
5.4.6.2. Colour intensity, colour tone and cielab colomeric indexes.....	55
5.4.6.3. Polymeric analyzed with bovine serum albumin (BSA) and bleaching by bisolphite.....	59
6. CONCLUSION.....	60
7. ATTACHMENTS.....	62
BIBLIOGRAPHY.....	64
SITOGRAPHY.....	67

Table's list

Table 1 – Identification of the 22 analyzed sample: type of company, “DO” consortium, other wines produced by the company

Table 2 - Vineyard surface and plant's age related to the 22 wines

Table 3 - Training system, pruning method related to the companies

Table 4 - % of Nebbiolo surface grown in the company, vineyard management in the companies

Table 5 - Grape and wine's analysis per company, destination of the wines

Table 6 – Results of company survey form about year of harvest, denomination of controlled origin, % Nebbiolo blend

Table 7 - Results of company survey form about crushing, fermentation, yeast inoculum, tank, T° control

Table 8 - Results of company survey form about malolactic fermentation, inoculation, heat

Table 9 – Results of company survey form about fining, months' aging, aging container

Table 10 - Basic analysis of the wines (1)

Table 11 - Basic analysis of the wines (2)

Table 12 - Total polyphenols of the wines (intensity of colour grows with the concentration)

Table 13 - Total flavonoids of the wines (intensity of colour grows with the concentration)

Table 14 - Total and monomer anthocyanins of the wines (intensity of colour grows with the concentration)

Table 15 - Proanthocyanidins of the wines (intensity of colour grows with the concentration)

Table 16 - Vanillin reactive flavanols of the wines (intensity of colour grows with the concentration)

Figure's list

Figure 1.- Cultivars in genetic relationship with 'Nebbiolo' and their cultivation area (Schneider et al., 2006)

Figure 2.- Map of the DOC/DOCG of Nebbiolo wines in North-West of Italy

Figura 3.- Coordinates CIELab e colorimetric indexes (Ozguven & Ozcelik, 2013, Tang et al., 2015, <https://www.fulviovilla.com/tag/cmyk/>)

Figure 4.- Places and altitudes of the considered vineyards (<https://www.google.it/maps>)

Figure 5 - Picture of sample No. 5 during the analysis

Figure 6 - Picture of sample No. 6 during the analysis

Figure 7 - Color intensity of the wines

Figure 8 - Coloring tone of the wines

Figure 9 - Values of L*, a* and b* of the wines

Figure 10 - Anthocyanidins monomers, LPP, SPP of the 22 samples

Figure 11 - Survey form, description of the company

Figure 12 - Survey form, description of the winemaking technique and the equipment used

1. INTRODUCTION

The characterization of a viticultural variety from the historical, geographical, phenological and oenological point of view is an ancestral interest of the human race.

Piedmont is an Italian region rich in *Vitis Vinifera* cultivars with almost millennial roots; Nebbiolo is the most important example.

Nebbiolo is a red grape variety, whose place of origin and etymology are uncertain. In addition to having more synonyms, the history of the name is intertwined with two different theories that have become legend: one linked to the name "fog", attributed to it with its very rich bloom on the berries, which would make them "clouded", the other hypothesis would be connected to the fog present during the first months of autumn, when the Nebbiolo is harvested due to the typical delayed ripening of the grapes (Dalmaso et al., 1962), compared to other Piedmontese red berry varieties.

The vine found optimal climatic and soil conditions in the western sub-alpine arc, in order to grow and give rise to wines of great entity, elegance and complexity. Starting from the most well-known areas such as Langhe and Roero, famous throughout the world for the prestigious Barolo and Barbaresco (100% Nebbiolo grapes), passing through the Asti area, and then crossing the doors of the capital Turin, from which the historical documents report the first evidence of cultivation of the "Nebbiolo" (Pietro de' Crescenzi, 1305). Thus we reach Canavese, north of Turin, where the training system changes, along with the climate and the type of soil and then, in North Piedmont, in the province of Novara, an area where Nebbiolo acquires excellent nuances under the name of Ghemme, Boca and Gattinara.

Finally two areas, completely different for altitude and landscape from the previous ones, are Valle d'Aosta and Valtellina (Lombardy), where also the name attributed to the vine has changed: respectively "Picotendro" in Valle d'Aosta and "Chiavennasca" in Valtellina. Recently, the presence of this variety has also been found in other parts of the globe such as Australia, New Zealand, Uruguay, Chile and Mexico for a total cultivated world of about 6000 hectares (www.aislombardia.it).

Regarding the protection of the product, 26 "Denominazione d'Origine Controllata" and "Denominazione d'Origine Controllata e Garantita" (DOC and DOCG) have been established, which for the policy document must be represented by at least 85% of Nebbiolo in the wine.

The Nebbiolo grape vinification is varied: in red, with a variant in Sforzato di Valtellina, sparkling rosé, sparkling white.

After a historical excursus, we will read about the viticulture and enology of Nebbiolo in the province of Turin and in the lower Valle d'Aosta analyzed in the experimental part of the thesis.

The wine samples were supplied directly by the companies after having submitted to them a company survey form that deals with the company's viticulture and oenology of the sample in question. What my thesis will focus on is the characterization of the phenolic profile of the coloring matter of Nebbiolo wine.

2. LITERATURE RIVIEW

2.1. The history of Nebbiolo

The first evidence of the Nebbiolo grape date back to 1268, where it is told of its cultivation around Rivoli.

Shortly afterwards, in 1300 in Asti, Petrus Crescentii with his "Opus Ruralium Commodorum" talks about the grape variety praising it: *"There is a kind of black grape that they say Nubiolum, which is not pleasant to eat, and is admirably vinous, it wants fat and dull soil and fears shade very much, it soon germinates and makes an excellent wine, which can be preserved and is very powerful. He must not stay beyond one or two days [to ferment] with his pomace; and this is held in best honor in the city of Asti and its surroundings."*(Schneider, 2017).

Stanislao Cordero of Pamparato, together with the travelers of the "Via Francigena", at the end of 1200, attest to the presence of the cultivar in the region.

In fact, along the way, the monasteries hosted not only the pilgrims but also the vines both for commercial and religious purposes, spreading Nebbiolo also in Val d'Aosta.

In 1495, the statutes of the municipality of La Morra recognize the grape variety as precious and to be protected.

One hundred years later, Gian Battista Croce, jeweler of the Royal House of Savoy, spoke of Nebbiolo as "queen of black grapes" (Berta et al., 1997).

The 1799, thanks to Count Giuseppe Nuvolone (head of the Agricultural Society of Turin), marks the beginning of the search for sub-varieties, which will later be defined as clones and will be those approved for cultivation in the Albese: "Lampia", characterized by a grape of small dimensions, "Michet" of blue-violet colour and "Rosé" the most unripe of all.

In the end of 1800, the arrival of phylloxera and downy mildew caused a change in viticulture: it was preferred to cultivate more resistant and fruitful vines than the *"noble and thorny"* Nebbiolo that remained in the Langhe area where, in 1730, is already mentioned the majestic "Barolo", in a correspondence between the English and Savoy.

During the whole of the 19th century, Barolo signed the strong points of its characteristics that are still famous throughout the world today: it is produce in dry way, full-bodied wine

with very long aging periods. The metamorphosis that leads to the present day the majesty of this wine is to be attributed to Carlo Alberto of Savoy in Verduno and the Falletti marquises in Barolo, who in close contact with the experts and the French aristocracy, include the winemaking techniques from across the Alps .

Fundamental and necessary figures in this transformation, thanks above all to their avant-garde vision, are General Paolo Francesco Staglieno and Camillo Benso Conte di Cavour. Mainardi, in 2004, reports the words of the general / winemaker Staglieno who, about Nebbiolo, expresses the need to produce a dry wine and predicts the four years of aging to reach "*their perfect goodness*" (Mainardi, 2004).

The particular attention of Count Camillo Benso towards agriculture and its subsequent appointment as minister of the same in 1850, allows Nebbiolo to be marketed in European markets.

The Piedmontese '800, seems to give a lot of importance to this vine, both for the method and the perseverance of the wine producers of the Langhe territory and for the oenological formation that is made possible by the birth of the oenological school in Alba in 1881 (Gerbi, 2016). Education in the continuous field, when in 1901 a farm was opened, currently called "*Ampelion*", also in Alba, which now houses the headquarters of the university course of Viticulture and Enology of Turin (Gerbi, 2016).

The long and complex history of Nebbiolo, has intrigued men of all times, to study the viticultural and oenological characteristics, up to the present day, where experiments in this regard have not stopped.

2.2. The origin of Nebbiolo

As can be seen from the previous point, the history of Nebbiolo has been well defined thanks to the numerous testimonies. You don't get the same luck, as far as genetic origin is concerned. Despite being one of the oldest vines in Italy and many abroad having ventured into its cultivation, Nebbiolo is deeply rooted in Piedmont and in the Alpine arc mentioned in the introduction. This is due to its particular behavior defined by the Americans "*fussy*" (Bettiga, 2003), to its susceptibility to training system, to the cultural practices attached to it and to the climate itself (in fact, a need for cold and temperature excursions regulated between day and night is necessary to be able to form aromatic precursors). Its vegetative and oenological characteristics agree with the appellative to be "*fussy*": it presents a wild and obstinate vigor, a discontinuous yield, the composition of the grapes appears unbalanced, in fact the harvest takes place only when the phenolic maturity is complete. In winemaking it originates products with a high alcohol content,

naturally poor in extractable anthocyanins that go against particularly rich, rigid and astringent tannins.

The great variability strictly linked to vintages, the need for a more in-depth clonal selection and the study of the territory of origin, the evolution and relationships of Nebbiolo, lead more and more to a genetic approach.

For over a decade (precisely 2004 and 2006), Professor Anna Schneider has conducted a lot of research in this regard; four sub-varieties were analyzed, "Bolla", "Lampia", "Michet" and "Rosé", comparing them with other Italian and European cultivars and a very complex picture emerged through the examination of the nucleic DNA.

The genotype "*Lampia*" is identical to the "*Michet*" and to the synonyms "*Bolla*", Picotendro and Chiavennasca, instead the genotype "Rosé" different from these sub-varieties (Schneider et al., 2006). However, there are cultivars, very close genetically to the vine under analysis, such as Freisa, which is cultivated throughout Piedmont, which shares 58 microsatellite loci thus showing a first degree relationship (Schneider et al., 2004). Another example is the Bubbierasco, an old vine from the Saluzzo area, which shares 23 microsatellite loci with Nebbiolo, which is referred as the direct parent, along with the above-mentioned, Bianchetto di Saluzzo.

The situation is complicated, since seven varieties of northern Piedmont and of these seven, six are native to the Valtellina are also closely related to the queen of red grapes raising the probability of being either "parent / child" or "brothers".

So, it is thought that the "Chiavennasca", so called Nebbiolo in Valtellina, is the starting point and that the original environment is just the Valtellina, but with current knowledge, it remains a probable but not confirmed hypothesis. The inexorable passage of time, does not allow us to become aware if Nebbiolo is progeny or parent and even less what could be the crossings due to the continuous evolution of the variety.

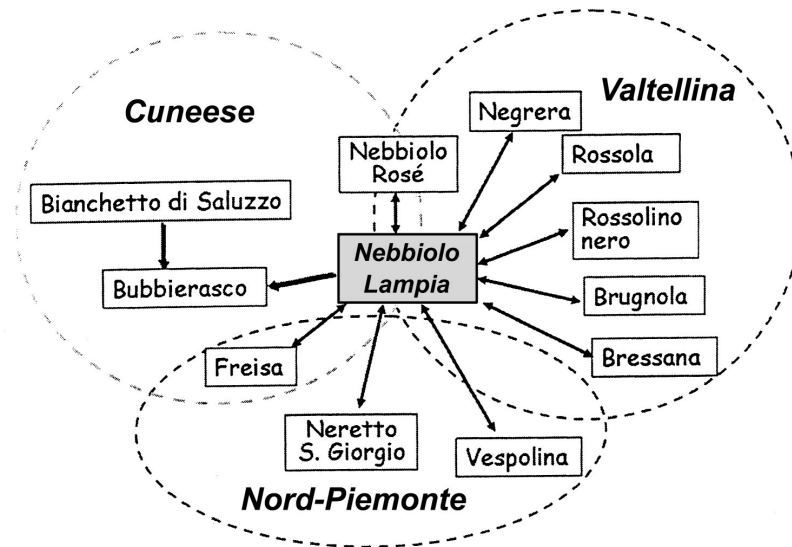


Figure 1.- Cultivars in genetic relationship with 'Nebbiolo' and their cultivation area. (Schneider et al., 2006)

2.3. Landscape and architecture of Nebbiolo

The areas taken into consideration in this experimental thesis start from the province of Turin, more precisely from the Canavese, along the road that leads to the lower Valle d'Aosta to reach Arnad.

Along this road a centuries-old path branches off: the "*Via Francigena*". This artery of medieval origins, linked Canterbury with Rome, to the Apulian ports and was rediscovered in the last decades by travelers interested in the "paths" of all Europe.

Many of the wineries treated in the area host this street right in the middle of the vineyards, and represent a point of rest and visit for travelers.



Figure 2.- Map of the DOC/DOCG of Nebbiolo wines in North-West of Italy

Traveling from South to North, the first area we meet is the Canavese area. This area is characterized by a very particular viticultural and enologic variety; the land is distinctly different from the areas of the "great Nebbiolos", such as Langhe and Roero, the origin is morainic tending to rocky in the whole area of the "Serra" (glacial amphitheater of the Quaternary era with a straight appearance) and the surrounding area.

The environment is known for a very rare method of vine cultivation: the pergola, here called in dialect "tòpia", in the most steep areas on terraces with dry stone walls. This system consists of a series of vertical poles, of wood or stone, supporting a scaffold positioned horizontally, called roof. On this scaffolding will be fixed canes. This type of architecture is shared throughout the area, up to the Valle d'Aosta and is the symbol of viticulture (later called heroic with right cause) and of the Canavese area itself.

The CERVIM, Center for Research, Studies, Safeguard, Coordination and Enhancement for Viticulture mountain, is an international organization born with the specific task of promoting and safeguarding heroic viticulture and for this reason defines its characteristics: the slope of the land must be higher than 30%, or the altitude above 500 meters asl, or the viticultural systems is positioned on terraces and steps and finally viticulture of small islands is also defined heroic. Being able to define a "heroic" wine goes to add a piece to

the history of the wine itself and shows all the dedication and effort of a vineyard so bristly but incredibly fascinating.

The terraces, very similar to those visible in the Douro Valley, in Portugal, do not have a standard size and go as far as micro-terraces, as the steep slopes rise between rocks eroded by glaciers. As "Cantina Produttori Nebbiolo di Carema" writes in the official website, in the "topiary architecture" section: "*These terraces are made up of alternating dry-stone walls with support functions, and fertile moraine land transported from the valley floor.*", we understand the mandatory need to build dry stone walls to contain the effects of debris carried by the melting of pre-existing glaciers.

Along the road to the Valle d'Aosta, the *tòpia* takes on different forms: in Canavese we find more the presence of wooden support poles, from Settimo Vittone, Carema to the Valdostana area on the coasts towering truncated-conical lime and stone pillars. On these columns, called "pilun" in dialect, are placed stone disks, where the racks supporting the vine shoots rest.

In addition to the landscape majesty and the solemn scenic effect, all these blocks of stone, both of the walls and of the pylons, play a fundamental role: they accumulate solar heat, then release it during the night, to cushion the rigid night mountain climate. However, it must be taken into consideration that with climate change in recent times, this function is less and less necessary.

2.3.1. The abandoning of pergolas and vineyards

During on-site visits to the companies, carried out for taking the samples and technical data sheets, I was able to observe an increasing abandonment of both the pergola and the vineyards themselves.

The maintenance costs of stone supports, worked with chisel and hammer, dry stone walls and terraces, lead wine growers to increasingly choose the conversion of vines to the espalier method. All this goes to the detriment not only of the grapes, which with the increase in temperatures, no longer benefit from the cooler microclimate of the *tòpia*, but also from the landscape point of view, the charm of a legacy handed down for centuries is lost, which represents the identity of a territory, in favor of the mechanization of the vineyards.

It is precisely this mechanization and its difficult attainment, in conjunction with the aging of the peasant population that transmits viticulture, leads to an even more extreme event: the total abandonment of the vineyards. It follows a total overgrowth of the terraces and reforestation, causing hydrogeological instability.

However, many producers resist the difficulties of this amazing landscape, characterized by a rare viticultural architecture, cultivating Nebbiolo on pergolas, producing wines, unique in their kind.

2.4. Phenolic profile and Nebbiolo oenology

As previously mentioned, Nebbiolo is one of the "noble" vines of north-west Italy and gives rise to great wines suitable for long aging.

When a picture is made of the scientific notions regarding the polyphenolic characteristics and the wine-making techniques, there are not many studies and most concern Barolo (Gerbi et al., 2000), Barbaresco (Ubigli et al., 1991) and on areas of the Langhe and Roero. This is certainly due to the latest investments to protect and incentivize, and to the exponential economic merit that these wines have acquired since the late 1980s, thanks also to a generation of Langaroli farmers who have revolutionized the concept of Nebbiolo: the "Barolo boys".

History and time have brought not only the notoriety of the vine, but thanks to scientific researches there is a greater awareness of work in the vineyard, since Nebbiolo is a late maturing variety and not malleable at all pedo-climatic conditions.

As demonstrated in previous studies, unlike international varieties such as Merlot and Cabernet (Di Stefano et al., 2003), Nebbiolo has poor ability to synthesize anthocyanins, with concentrations in the grapes of 400-600 mg / kg of grapes (Bosso et al., 2004).

In order to reach the maximum levels of the product, thus favoring more vivid colors and loads, those who work in the vineyard have decided to introduce studied techniques, such as better leaf management and cluster thinning (Guidoni et al., 2002).

Furthermore, we know that the environment and the climate affect the color of Nebbiolo in an incisive manner; the synthesis of anthocyanins is facilitated by the brightness, but high temperatures cause their degradation (Di Stefano et al., 2003) and this last phenomenon is more and more frequent due to the raising of the average temperatures due to climate change.

2.4.1. Phenolic profile of Nebbiolo

It has been observed for some time that Nebbiolo is a variety with a low anthocyanin content and abundant in tannins, with a not particularly resistant skin, even if the cultivation environment, whether mountain or hilly, influences the hardness of the skin and extractability of anthocyanins during maceration (Rolle et al., 2006).

The anthocyanin profile of the Nebbiolo grapes is singular, in fact it is characterized by almost 50% of peonidine, the bis-substituted anthocyanin, malvidin represents 15-35% of the total and finally another anthocyanidin present in the vine is cyanidin with a percentage that ranges between 8 and 17% (Gerbi et al., 2004). The percentages are guided by multiple factors that have an intrinsic variability: the vintage, the climate, the chosen clone, the maturity of the grapes, and the area where the vineyards are present, as in example the south of Piedmont or mountain areas like Valle d'Aosta or Valtellina.

Going deeper into the nature of the representative anthocyanins of Nebbiolo, it is known as the peonidin, an antocianidin bis-substituted with two neighboring substituents on the side ring, it is easily extractable in aqueous solution, during the maceration when the first fermentation has not yet consumed all the oxygen present that would cause an easy and rapid oxidation (Gerbi et al., 2002). Another easily extractable anthocyanidin, as it is oxidizable, is the bis-substituted cyanidin, with a high ability to spread in the crushed. Unlike the first two, malvidin, tris-substituted, is more stable against oxygen.

As mentioned above, the total concentration of anthocyanins in the grapes is low compared to other red berry varieties. This contrasts with the high concentration of tannins that are extracted in an alcoholic solution, when the alcoholic fermentation has already started for days, going to establish at the end of the same an anthocyanin / tannin ratio of 1 to 7/1 to 10, therefore unfavorable to the color of the wine (Gerbi et al., 2004). The proanthocyanidins are present for 59% in the seeds, 21% in the petioles, 20% in the skin; however, as regards Nebbiolo, the presence of tannins is greater in the skin than in the seeds (Cravero et al., 1992). Tannins of the seeds have a lower degree of polymerization than the tannins of the skin and are more bitter; they also have a certain "grado di galleizzazione" (the greater the latter, the greater the astringency). The proanthocyanidins present in the skin and in the grape seeds, apart from differing in the degree of polymerization, differ in the structural units. The proanthocyanidinic tannins of seeds have procyanidinic units, those of the skins also have prodelfinidinic units. The degree of polymerization, the number of monomers involved in the copolymer, is greater in proanthocyanidins contained in the skin than in those contained in the seeds. The tannic properties of Nebbiolo are high, compared to other varieties, such as international ones, for example. Its grape seeds possess a high percentage of flavanes; they have a low molecular weight so it is necessary for the grapes to reach technological maturity, so that the flavans themselves are not released in the medium, which would give rise to an astringent and rough product on the palate. However, the long macerations favor the extraction of tannins from the skin, a distinctive trait of Nebbiolo wine, without going to excessively extract the tannins of the seeds, which are too astringent (Gerbi et al., 2006).

2.4.2. Vinification of Nebbiolo

With a greater knowledge of the polyphenolic framework and the difficulties in the vinification of Nebbiolo compared to international varieties, the producers sought methods to be able to reduce the supply of oxygen before alcoholic fermentation. This is due to the drastic lowering of cyanidin and peonidin, with an unequivocal raising of the maldivina, which constitutes the "young" Nebbioli (Gerbi et al., 2004). Consequently, to be able to stabilize, extract as much as possible the bis-substituted anthocyanins, to arrive at wines with a more intense coloring intensity and to subtract the more astringent tannins and make them polymerize those of the skins tied to the polysaccharides of the cell walls, is due to 3 elements: the skill of the winemaker, the winemaking technique and the grapes must be ripe (Gerbi et al., 2006).

To meet these needs, the studies proposed different maceration procedures to favor extractions. For example the *délestage*, a technique that consists in emptying the fermenter of all the liquid, transferring it to a tank temporarily, letting the hat of the skins go down to the bottom of the tank, then pump over the must on the solid parts (Bosso et al., 2004).

Another technique is the cold pre-fermentation maceration, where the freshly pressed grapes are kept at 10 ° C (in some cases we find even colder environments), for a minimum of 24 hours up to a maximum of 96, restore the "ambient" temperature of 20 ° C to start the fermentation with a "pied de cuve", breaking the hat with punching down and pumping over (Bosso et al., 2007).

This technique, as explained by Di Stefano and colleagues in 2003, brings an extension in the extraction of anthocyanins, when the danger of red-ox reactions has been lowered and during the extraction of tannins from seeds and skins, so as to favor the anthocyanin-tannin polymerization, through acetaldehyde and ethyl bridge. Furthermore, results can also be obtained with regard to potassium bitartrate, since after alcoholic fermentation it is more stable, it will fall less, taking away a smaller quantity of color.

Another aspect to which attention must be paid during vinification, first of all from the temporal point of view, is the crushing-destemming. Nebbiolo is characterized by a green stem at the time of the technological maturity of the grapes, consequently a destemmer and crusher that leave within the crushed, little piece of stems and lacerate the skins instead of leaving them intact, leading to herbaceous scents and inadequate extraction of color (Gerbi et al., 2006). Therefore, it is right to concentrate in this phase of processing, not only with the care of the technicians, but also by investing in a machine that first de-stem, equipped with a very efficient beater shaft (given the resistance of the Nebbiolo

pedicel to detachment) and then press in order to have the grapes as clean and intact as possible.

2.4.3. The colour of Nebbiolo

2.4.3.1. Maceration and stabilization of the colour

As mentioned in the chapter on the phenolic profile, the low quantity of Nebbiolo coloring matter causes a deficit in the color of the wine and it is necessary to have greater precautions and techniques studied to preserve and maintain the anthocyanins present in the grapes originally (Cagnasso et al., 2001; Gerbi et al., 2002, 2004; Garofano et al., 2003).

A topic discussed at length is the maceration of Nebbiolo. Some producers have opted to shorten the days of maceration on the skins to prevent loss of colour and to avoid the excessive extraction of flavanols; other companies more linked to tradition have continued with longer macerations, in order to extract all the tannins that will go then to polymerize with the anthocyanins, so as to stabilize the color (Gerbi et al., 2006).

Maceration remains the fundamental process in which the initial and final color of Nebbiolo wine is determined. Speaking of the coloring matter of a wine, the contribution of the total anthocyanins to the red color is given by the sum of free monomer anthocyanins and those linked with tannins. The monomer anthocyanins are used to study the indexes of color stability. With the vinification, free anthocyanins, in part either are not extracted, or are lost or polymerized with tannins. The loss of anthocyanins is due to the loss of monomer anthocyanins; it is necessary to favor the tannin-anthocyanin bond directly (Somers, 1971) or with the ethanal that creates a bridge between the two (Timberlake, Bridle, 1976) or with secondary metabolites of microorganisms present in the wine (Bakker, Timberlake, 1997; Benabdeljalil et al., 2000), in order to preserve color as much as possible. We find the greatest loss at the time of racking; this depends on the extractability of the anthocyanins, hence variety dependent. Nebbiolo at this stage has a 30% drop in free anthocyanins: of which 25% is because it is not possible to extract them and the remaining 5% oxidizes, are absorbed by yeasts and precipitate with tartaric acid (Cagnasso et al., 2007). After the racking, the value of the anthocyanins decreases, this is also due to malolactic fermentation and tartaric stabilization itself (Ribereau-Gayon et al., 2005).

After the first year of life of a wine, the monomer anthocyanins have a percentage of 52%; consequently it is necessary to take preventive action to ensure that these free anthocyanins, instead of falling, bind to tannins. For this reason some solutions such as

micro-oxygenation and aging are important as they increase the proportion of monomer anthocyanins that binds (Ribereau-Gayon et al., 2005).

Another occasion to which particular attention must be put is at the time of bottling; we must know the value of the anthocyanins monomers because they could give precipitates in the bottle in the form of crystalline deposit: the salts of tartaric acid. If a powdery deposit is found it is because the wine has been bottled too young and not yet stable. It is therefore necessary to stabilize the free anthocyanins, so that they can be found in the wine even after a possible aging (Ribereau-Gayon et al., 2005). The use of wood in aging and its supply of oxygen through the staves of the barrels / casks, helps to establish links between the anthocyanins and the tannins of the grapes, through ethyl bridges (Moutounet et al., 2003).

The role of the oenologist is to soften the natural decrement of the total anthocyanins by making the gradual and content possible, passing between the various steps of drawing off, malolactic fermentation and tartaric stabilization. Certainly in enology topic, as far as Nebbiolo is concerned, a lot of research still needs to be deepened and studied, given the variety of climate, the environment in which the grape is grown.

2.4.3.2. Colorimetric indexes

The first sensory organ that comes into activity when approaching a wine is the sense of sight, in fact color is the first parameter that is evaluated at the time of tasting, this was also highlighted in the works of Brochet, 2001, Brochet and Dubordieu, 2001 and Morrot and colleagues, 2001.

In the sensory analysis of color, perceptions are manifold: physical, psycho-physical and psychological. From these start neural stimuli that are clearly interpreted subjectively. In 1997, Professor Cagnasso studied tristimulus colorimetry to describe color detection, with objective parameters.

The visible absorption spectrum of red wines ranges from 350 to 600 nm. Each wine responds with a different spectrum and through indexes we can understand how color is made. The coloring intensity is given by the sum of the absorbances at 420nm + 520nm + 620nm. The higher it is, the more intense the color, but it does not indicate which color it is. The coloring shade is the ratio between absorbance at 420nm and 520nm; the higher it is, the more the color of the wine is penalized because it will tend towards the orange hues that absorb at 420nm. The CIELab coordinates, uniquely identify the color through coordinates; these are L *, a *, b *. L stands for brightness and goes from 0 to 100, the higher it is, the clearer it is; the more a * has a low value, the more it will tend to green, the

higher it is, the more the color will be red. Finally $-b^*$ will correspond to the color blue and $+b^*$ to the color yellow.

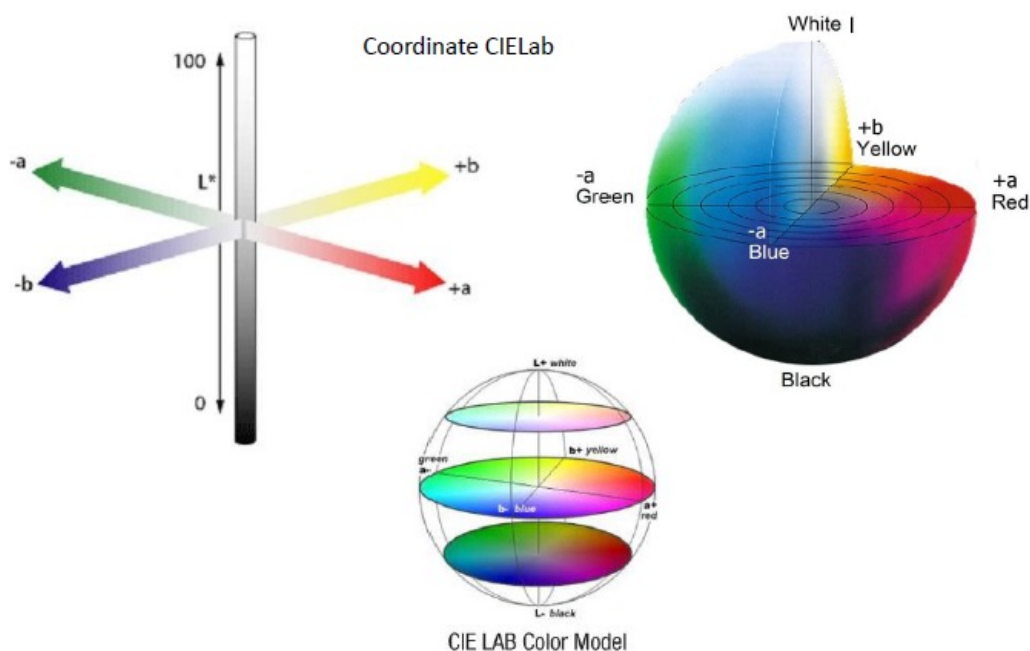


Figure 3.- Coordinates CIELab e colorimetric indexes (Ozguven & Ozcelik, 2013, Tang et al., 2015, <https://www.fulviovilla.com/tag/cmyk/>)

3. AIM OF WORK

The aim of the work was to acquire more information on the state of the art of Nebbiolo in the province of Turin and the lower Valle d'Aosta so as to increase the knowledge on the vine by expanding the research area. In particular, frame the adopted winemaking methods and the composition of the wines obtained, with particular reference to the phenolic component and its effects on the color of the wines. In this phase of the work, the samples were compared with more research carried out in more known areas such as Langhe, Monferrato and Roero. Furthermore, the aim is to have a wider knowledge of an area, which was previously ignored, but for some years now, has returned to attention for a revaluation of the territory.

4. MATERIALS AND METHODS

4.1. Company survey form

A total of 22 wine samples were collected and examined in this work, of which 4 Carema DOC, 2 Pinerolese Rosso DOC, 2 Vallée d'Aoste Arnad-Montjovet DOC and 13 Canavese Nebbiolo DOC, 1 table wine with Nebbiolo grapes, of vintages between 2015 and 2018. The sample collection period lasted about 3 weeks, between February and March 2019.

Having considered a range of 4 years, the wines were acquired both directly from wooden tanks / stainless steel tanks, suitable for fermentation and aging, and other already bottled for sale.

There are 20 participating companies, part of the province of Turin, Biella and the lower Valle d'Aosta.

All the elements analyzed consist of a minimum percentage of 85% of Nebbiolo, some of which are wines that follow product specifications (Cavanese Nebbiolo DOC, D.M. 07.03.2014, G.U. 295 - 20.12.2011, Carema DOC, D.M. 07.03.2014, G.U. 295 - 20.12.2011, Valle d'Aosta DOC, D.M. 30.11.2011, G.U. 183 - 06.08.2008). The wines were taken directly from the company, after having submitted to the persons in charge two different survey forms: one, concerning the description of the company from the general and viticultural point of view, the other, concerns the sample , the winemaking technique and the equipment that was used.

Table 1 – Identification of the 22 analyzed sample: type of company, “DO” consortium, other wines produced by the company.

N° SAMPLE	COMPANY	CONSORTIUM OF PROTECTION	WINE TYPE PRODUCED IN COMPANY
1	Private company	Consorzio per la Tutela e la valorizzazione dei vini DOCG di Caluso e DOC di Carema e Canavese	BARBERA CANAVESE, CANAVESE ROSSO, CANAVESE NEBBIOLO
2	Private company	No	Nebbiolo e Erbaluce
3	Private company	Consorzio per la Tutela e la valorizzazione dei vini DOCG di Caluso e DOC di Carema e Canavese	Erbaluce di caluso docg still, spumante and passito, Canavese rosso, Canavese nebbiolo
4	Private company	Consorzio di Tutela Vini Doc Pinerolese	Pinerolese Ramie, Pinerolese doux d'Henry, Pinerolese Barbera, Pinerolese Rosso Nebbiolo, Pinerolese Dolcetto, Pinerolese Bonarda
5	Private company	No	Canavese Nebbiolo, Canavese Barbera, Canavese Bianco, Spumante
6	Private company	Consorzio per la Tutela e la valorizzazione dei vini DOCG di Caluso e DOC di Carema e Canavese	Erbaluce docg (still, passito e spumante), Canavese Rosso doc e Canavese Nebbiolo Doc
7	Private company	Consorzio per la Tutela e la valorizzazione dei vini DOCG di Caluso e DOC di Carema e Canavese	Erbaluce di caluso, Caluso spumante docg, Canavese rosso, Caluso passito
8	Private company	Consorzio di Tutela Vini Doc Pinerolese	Pinerolese Ramie, Pinerolese doux d'Henry, Pinerolese Barbera, Pinerolese Rosso Nebbiolo, Pinerolese Dolcetto, Pinerolese Bonarda
9	Private company	No (vivalb)	Valle d'Aosta DOC Nebbiolo, Valle d'Aosta Doc Donnas
10	Private company	No (vivalb)	/
11	Cooperative winery	Consorzio per la Tutela e la valorizzazione dei vini DOCG di Caluso e DOC di Carema e Canavese	Carema Doc e Riserva, Canavese Nebbiolo Doc, Canavese Rosso Doc e Canavese Rosato Doc, Red wine, White wine, Spumante Nebbiolo
12	Private company	No	Carema Doc e Canavese Nebbiolo
13	Private company	No	Carema Doc
14	Private company	Consorzio per la Tutela e la valorizzazione dei vini DOCG di Caluso e DOC di Carema e Canavese	Erbaluce di Caluso (spumante, fermo, passito), Carema Doc
15	Cooperative winery	Consorzio per la Tutela e la valorizzazione dei vini DOCG di Caluso e DOC di Carema e Canavese	/
16	Cooperative winery	Consorzio per la Tutela e la valorizzazione dei vini DOCG di Caluso e DOC di Carema e Canavese	/
17	Private company	No	Canavese Bianco Doc, Canavese Rosato, Canavese Nebbiolo, Canavese Barbera, Red wine, Spumante rosè brut
18	Private company	No	Canavese Rosso Doc e Canavese Nebbiolo Doc
19	Private company	Consorzio per la Tutela e la valorizzazione dei vini DOCG di Caluso e DOC di Carema e Canavese	Erbaluce di Caluso Docg, Erbaluce di Caluso spumante Docg, Passito, Canavese Rosso Doc, Canavese Nebbiolo Doc
20	Private company	Consorzio per la Tutela e la valorizzazione dei vini DOCG di Caluso e DOC di Carema e Canavese	Erbaluce Docg, Canavese Rosso Doc, Canavese Nebbiolo Doc, Bulk wine
21	Private company	No	Canavese rosso, Canavese Nebbiolo, Others
22	Private company	Consorzio per la Tutela e la valorizzazione dei vini DOCG di Caluso e DOC di Carema e Canavese	Canavese Nebbiolo Doc, Freisa, Chatus, Spumante Nebbiolo

4.2. Chemical analysis

Subsequently, chemical analysis were carried out on the various samples for investigative purposes and in-depth analysis on the Nebbioli of the treated area.

The basic analysis developed are:

- ethanol (% vol.)
- residual sugars (g/L)
- total acidity expressed in tartaric acid (g/L)
- volatile acidity expressed in acetic acid (g/L)
- pH
- malic acid (g/L)
- lactic acid (g/L)
- tartaric acid (g/L)
- total dry extract (g/L)
- glycerol (g/L)
- ashes (g/L)

These were processed by FTIR analysis technique with the WineScan produced by FOSS, Hilleroed, DK.

The analysis on total polyphenols were carried out with the Folin-Ciocalteu indexes and the results were expressed in mg/L (+) - catechin (Di Stefano et al., 1989). All phenolic compounds contained in wine are oxidized by Folin-Ciocalteu reagent. This reagent is formed from a mixture of phosphotungstic acid, $H_3PW_{12}O_{40}$, and phosphomolybdic acid, $H_3PMo_{12}O_{40}$, which, after oxidation of the phenols, is reduced to a mixture of blue oxides of tungsten, W_8O_{23} , and molybdenum, Mo_8O_{23} . The blue coloration produced has a maximum absorption in the region of 750 nm, and is proportional to the total quantity of phenolic compounds originally present.

The phenolic compounds examined are:

Total flavonoids, mg/L (+) - catechin (Di Stefano, 1989).

Total anthocyanins, mg/L malvidin 3-O-glucoside chloride and anthocyanidins monomers, mg/L malvidin 3-O-glucoside chloride (Di Stefano et al., 1989). This method exploits the absorption of anthocyanins in an acid-alcoholic environment, and allows us to evaluate the quantity of coloring substances present in the sample.

Proanthocyanidin index, mg/L cyanidin chloride (Di Stefano et al., 1989). This index is useful for quantifying the condensed tannins present in the samples in question, whose excessive presence determines the sensation of astringency. To evaluate the amount of Proanthocyanidins present are colored in an acid and hot environment, correcting

the results obtained with the graphic method to deduct the contributions of the brown polymers that are formed from catechins.

Flavanols reactive to vanillin, mg/L (+) - catechin (Di Stefano et al., 1989). Vanillin is a substance capable of reacting with the 6 and 8 positions of flavanols: it is therefore possible to quantify the presence of free flavanols in the sample examined, responsible for the scents bitter if in excessive quantity.

Fractionation and color index of wines at pH 0, (Versari et al., 2008), to detect monomer pigments, sensitive and not to SO₂, expressed as a percentage. The spectrophotometric analysis of the polymer pigments of red wines is based on two assumptions: SO₂ almost totally discolours the monomer and anthocyanins only partially the polymeric pigments; the color of the polymer pigments is less sensitive to changes in pH.

As for the chromatic properties we obtained the coloring intensity as the sum of the absorbances at 420 nm, 520 nm, 620 nm, the coloring shade as the ratio between the absorbance at 420 nm and 520 nm (OIV-MA-AS2-07B. OIV, 2016) and finally the CIELab indexes, with the determination of the clarity (L*), of the red-green component (a*) and of the yellow-blue component (b*) (OIV-MA-AS2-11. OIV, 2016).

The last analysis was the measurement of the polymeric pigments of wines using a protein precipitation (precisely the bovine serum albumin, BSA), combined with whitening with bisulfite (Harbertson et al., 2003) expressed as a percentage.

5. RESULTS AND DISCUSSION

5.1. Company survey form - company description

5.1.1. Company, vineyard surface and wine type

It is not possible to study the correlation between plant density and analytical parameters of the wines because the vineyards are at different altitudes, with non certified propagation materials, produced in different years, with different methods. Also about statistical method we don't have repeatability during the analysis.

Out of 20 companies, 2 are cooperative wineries and the rest are private companies; this is also evident with regard to the vineyard surface, since both are those with more hectares, thanks to the transferring partners (sample n ° 11 = 19 hectares, n ° 15/16 = 150 hectares). The reality in the areas taken into consideration is characterized by micro-companies and small-medium companies; despite this, all the companies have opted to have a range of wines produced not exclusively focused on Nebbiolo (excluding only the company of the

sample n ° 13, which exclusively produces Carema DOC), but that goes to enhance typical wines of the place even in blend with minor grape varieties. In the area of Canavese and the surrounding area, the most cultivated grape variety, in addition to the “queen of black grapes” is Erbaluce, a white variety native to the area.

Table 2 - Vineyard surface and plant's age related to the 22 wines

N° SAMPLE	VINEYARD SURFACE (HA)	PLANT'S YEAR
1	1.3	10
2	1.5	From 5 to 100
3	13	From 5 to 50
4	5.5	25
5	0.9	From 1 to 12
6	8.5	From 1 to 100
7	4	From 10 to 60
8	5.5	25
9	2.5	From 10 to 80
10	15	From 10 to 30
11	19 (14 Carema doc)	100
12	1	From 1 to 80
13	0.8	From 3 to 50
14	6	From 20 to 60
15	150	From 2 to 70
16	150	From 2 to 70
17	6	From 9 to 60
18	2	From 4 to 40
19	2	From 12 to 45
20	3	>30
21	2.5	From 10 to 80
22	1.8	From 15 to 60

5.1.2. Location and altitude



Figure 4.- Places and altitudes of the considered vineyards (<https://www.google.it/maps>)

5.1.3. Training system and pruning system

As far as the training system is concerned, companies are divided into 3 main categories: 7 companies adopt row, as happens in southern Piedmont, where Nebbiolo is grown exclusively in row. 4 companies out of 20 maintain the typical and characteristic method in pergola and finally 9 out of 20 have both training systems, with different subdivisions. For sample 11, from the Carema area, it has a majority of "tòpia" for the cultivation of Nebbiolo; for another Carema company, sample n° 13, the use of the Pergola in the total company represents 30%, preferring row.

For all companies, both row and "tòpia" are pruned to Guyot. The training system will influence the growth of the plant, the bunch itself, the technological maturity and the phenolic one.

Table 3 - Training system, pruning method related to the companies

N° SAMPLE	TRAINING SYSTEM	PRUNING
1	Row	Guyot
2	Pergola	Guyot Pergola
3	Row	Guyot
4	Row	Guyot
5	Row	Guyot
6	Both	Guyot e Guyot Pergola
7	Both	Guyot e Guyot Pergola
8	Row	Guyot
9	Pergola	Guyot Pergola
10	Row	Guyot
11	Both (> Pergola Nebbiolo)	Guyot e Guyot Pergola
12	Both	Guyot e Guyot Pergola
13	Both (30% pergola 70% row)	Guyot e Guyot Pergola
14	Both	Guyot e Guyot Pergola
15	Both (60% pergola, 40% row)	Guyot e Guyot Pergola
16	Bow (60% pergola, 40% row)	Guyot e Guyot Pergola
17	Row	Guyot
18	Pergola	Guyot Pergola
19	Both (row x Nebbiolo)	Guyot e Guyot Pergola
20	Pergola	Guyot Pergola
21	Both	Guyot e Guyot Pergola
22	Row	Guyot

5.1.4. Nebbiolo grown in company

An interesting point to analyze is the percentage of Nebbiolo from each company on the total number of vines cultivated. Dividing into two sections, <50% and > 50%, there is a clear division between the 20 companies: 10 of these have a lower percentage of 50% in the overall company, which varies from 4% to 40% and the another half varies from 60% to 100% ownership. This shows how each company gives a different importance to the vine considered.

5.1.5. Vineyard management

Half of the samples (11) have a conventional vineyard management. 7 samples out of 22 opted for an integrated pest management practice. 3 samples are organic certified. Only sample No. 7 has both integrated pest management practices and organic in the company.

Table 4 - % of Nebbiolo surface grown in the company, vineyard management in the companies

N° SAMPLE	NEBBIOLO GROWN	VINEYARD MANAGMENT
1	25%	Conventional
2	60%	Organic
3	4%	Integrate
4	100%	Convenzional
5	40%	Integrate
6	20%	Organic
7	15%	Integrate and Biologic
8	100%	Convenzional
9	90%	Convenzional
10	60%	Integrate
11	80%	Convenzional
12	85%	Convenzional
13	70%	Convenzional
14	30%	Convenzional
15	40%	Integrate
16	40%	Integrate
17	60%	Integrate
18	80%	Integrate
19	15%	Organic
20	15%	Convenzional
21	10%	Convenzional
22	70%	Convenzional

5.1.6. Grapes and wine analysis

Oenology and winemaking can support the success of a wine, mitigating pre-existing problems, but the quality of the raw material must be essential. Especially for wines considered medium-high / high range; consequently it is necessary to support the experience of the winemaker with the technological and phenolic maturation indexes. Precisely for this reason it is increasingly crucial to decide the best time to harvest the grapes, based on the analysis carried out, the weather and the sensorial analysis of the grapes.

Nebbiolo grapes produced in a mountain environment, as to be expected, at the harvest have high sugar concentrations, but lower than those of the grapes coming from hilly areas (Mannini et al., 2006).

All the companies involved, except one, do the analysis of the sugars on the grapes close to the harvest; 14 out of 20 analyze the total acidity (g/L), one of the crucial points of technological maturity. 5 companies control the pH value and only 4 companies out of the total analyze the phenolic content. For Nebbiolo it is fundamental to control phenolic maturity, given its low starting point, anthocyanin content and, on the contrary, high proanthocyanidin content (Gerbi et al., 2006). We must try to get as much as possible to

have a combined technological maturity with the phenolic one, so as to have an excellent starting material and only be able to enhance it during winemaking.

Equally important are the analyzes during alcoholic fermentation, with finished wine already stabilized from the protein and tartaric point of view, ready for bottling.

During the vinification, the lowering of the sugars was checked on all the samples except the sample n ° 12, thanks to the help of a mostimeter.

At the end of the fermentations, both alcoholic and malolactic, all the companies except the sample n ° 12, performed analyzes on the content of the wines of: ethanol (%vol), residual sugar (g/L), essential to check that the wine is dry and not have problems of further refermentations, total and volatile acidity (g/L), pH, malic acid (g/L), lactic acid (g/L); these last two are necessary to monitor if the malolactic fermentation has had interruptions during the process, or if it has been completed.

Another parameter that must be monitored, since the legal limit imposed is a maximum of 150 mg/L, is sulfur dioxide. 7 samples out of 22 perform analyzes on the content of sulphites in wine. 4 samples out of 22 control the content of residual metals and 4 out of 22 carry out analyzes on tartaric stability and protein stability.

5.1.7. Bulk/bottled

The marketing of the wine is divided into two modes: sale in bottles and bulk, taken directly from tanks dedicated to direct trade.

The realities examined, being small and linked to local buyers, use widely the "bulk" to distribute the production, with a peak by the company No.1 which reaches up to 70% of the total of its production.

Over the years there has been an evolution in the purchase of wine, tending more and more to prefer the bottled at the expense of "bulk". As we can see from the table 3, 8 companies out of 20 produce only the finished and bottled product (100% bottle) in favor of a better conservation, a delayed consumption and with a further possibility of export outside Italy. This leads us to think, that the attention to conservation and to having a finished product with a higher quality, is pointing towards the almost total abandonment of the "bulk".

Table 5 - Grape and wine's analysis per company, destination of the wines

N° SAMPLE	GRAPES AND HARVEST'S ANALYSIS	WINE'S ANALYSIS	BULK/BOTTLED
1	Sugar	Sugar, Foss	70% - 30%
2	Sugar, Acidity	Sugar, Foss	???
3	Sugar, Acidity	Sugar, Foss (SO2 and Copper)	10% - 90%
4	Sugar	Sugar, Foss	0% - 100%
5	Sugar, Acidity	Sugar, Foss	0% - 100%
6	Sugar, Acidity, pH	Sugar, Foss	20% - 80%
7	Sugar, Acidity, Nitrogen	Sugar, Foss, Tartaric and Proteic stability	0% - 100%
8	Sugar	Sugar, Foss	0% - 100%
9	Sugar, Acidity, pH, Phenolic maturation	Sugar, Foss	0% - 100%
10	Sugar, Acidity, Phenolic maturation	Sugar, Foss	20% - 80%
11	Sugar, Acidity, pH	Sugar, Foss	30% Bag in Box, 70%
12	No	No	0% - 100%
13	Sugar, Acidity	Sugar, Foss (SO2)	0% - 100%
14	Sugar, Phenolic maturation	Sugar, Foss	1% - 99%
15	Sugar, Acidity	Sugar, Foss (SO2 and Copper)	30% - 70%
16	Sugar, Acidity	Sugar, Foss (SO2 and Copper)	30% - 70%
17	Sugar, Acidity, Phenolic maturation	Sugar, Foss (metals)	0% - 100%
18	Sugar, Acidity, pH	Sugar, Foss	40% - 60%
19	Sugar	Sugar, Foss (SO2), Tartaric and proteic stability	0% - 100%
20	Sugar, Acidity	Sugar, Foss (SO2), Tartaric and proteic stability	30% - 70%
21	Sugar, Acidity	Sugar, Foss (SO2), Tartaric and proteic stability	50% - 50%
22	Sugar, pH	Sugar, Foss	20% - 80%

5.2. Company survey form - description of production technique and equipment used

5.2.1. Nebbiolo blend

All the wine samples are composed of at least 85% Nebbiolo, the sample No. 18 has a 95%, the numbers 9 and 13 have 90% Nebbiolo grapes and 17 samples out of 22 are characterized by 100% Nebbiolo. In the areas considered, the typical assembly varieties are: Neretto di Bairo, Ner d'Ala, Croatina, Vespolina, Chatus. These cultivars are generally used for their content of total anthocyanins higher than Nebbiolo, so that they can be integrated in vinification with a stronger red color (Gerbi et al., 2005). PER CITARE LA TABELLA SOVRASTANTE = CF. NUMERO TABELLA.

Table 6 – results of company survey form about year of harvest, denomination of controlled origin, % Nebbiolo blend

N° SAMPLE	YEAR	DENOMINATION OF CONTROLLED ORIGIN	% NEBBIOLO BLEND
1	2018	CANAVESE NEBBIOLO DOC	100%
2	2018	CANAVESE NEBBIOLO DOC	100%
3	2018	CANAVESE NEBBIOLO DOC	100%
4	2018	PINEROLESE ROSSO DOC	100%
5	2017	CANAVESE NEBBIOLO DOC	100%
6	2017	CANAVESE NEBBIOLO DOC	100%
7	2017	CANAVESE NEBBIOLO DOC	100%
8	2017	PINEROLESE ROSSO DOC	100%
9	2017	VALLÉE D'AOSTE ARNAD-MONTJOVET DOC	90%
10	2017	VALLÉE D'AOSTE ARNAD-MONTJOVET DOC	100%
11	2017	CAREMA DOC	100%
12	2016	CAREMA DOC	85%
13	2016	CAREMA DOC	90%
14	2016	CAREMA DOC	100%
15	2016	CANAVESE NEBBIOLO DOC	100%
16	2016	CANAVESE NEBBIOLO DOC	100%
17	2016	CANAVESE NEBBIOLO DOC	100%
18	2016	CANAVESE NEBBIOLO DOC	95%
19	2016	CANAVESE NEBBIOLO DOC	85%
20	2016	CANAVESE NEBBIOLO DOC	100%
21	2016	VINO DA TAVOLA	100%
22	2015	CANAVESE NEBBIOLO DOC	100%

5.2.2. Crushing

As already mentioned in the literature review, two of the first processing on grapes, such as destemming and crushing, are of fundamental importance for the future quality of the wine.

8 samples out of 22 before destemming and then crushing, 11 out of 22 first crushing and then destemming and finally only the samples n° 6, n° 12, n° 16 do only destemming. Many of the manufacturers have claimed to use a crushing-destemming, not for personal choice, but for economic issues, as they are already present in the company and the investments are not currently focused on replacing this machine.

5.2.3. Fermentation – Yeast inoculum

8 samples out of 22 do not inoculate the tanks with selected yeasts, and make spontaneous fermentations with the indigenous yeasts present on the grapes. In some wineries, given the longevity and numerous harvests carried out, it is no longer necessary to inoculate

selected yeasts, since the environment itself has the suitable conditions for the development of *Saccharomyces Cerevisiae*.

For case number 2, it's different, since the company is certified organic, with a biodynamic future; at the time of the interview the owner has precisely expressed the desire not to inoculate to make his Nebbiolo in the most natural way and without additives as possible. The remaining 14 samples were vinified with inoculum of selected rehydrated yeasts.

5.2.4. Fermentation – Tank

An important choice for those who produce wine is the fermenter used to make Nebbiolo. Thanks to previous studies and experience, I can say that the tank suitable for the most aseptic alcoholic fermentation, easily sanitized and controllable from the temperature point of view is certainly the stainless steel tank. 20 samples out of 22 use the stainless steel tank in the first part of the vinification. Only 2 samples use not only stainless steel but also other containers such as concrete tanks, tonneaux and barriques.

The producer of the sample n° 11 has explained that, given the substantial volumes produced, he wants use all the tanks present in the company and that the concrete being coated inside, has no releases to the wine. The producer of the sample No. 7 opts to divide the vinification into different tanks, choosing the use of tonneau and barriques, both for a sensory factor, and for color stabilization.

5.2.5. Fermentation – T° control

The third fundamental point during winemaking is the control of temperatures during alcoholic fermentation (Riberau-Gayon et al., 2005). Out of the total of 22 samples, only 2 do not control them; both companies, however, have only been born year ago and the economic possibilities are reduced.

In all the other 20 samples, the C° are monitored thanks to glycol plates, insulated and semi-insulated tanks.

Table 7 - Results of company survey form about crushing, fermentation, yeast inoculum, tank, T° control

N° SAMPLE	CRUSHING	FERMENTATION - YEAST INOCULUM	TANK	T° CONTROL
1	Destemming - Crushing	No	Inox	Yes
2	Crushing - Destemming	No	Inox	No
3	Crushing - Destemming	Yes	Inox	Yes
4	Crushing - Destemming	No	Inox	Yes
5	Crushing - Destemming	Yes	Inox	Yes
6	Destemming	No	Inox	Yes
7	Crushing - Destemming	No	Inox + Barrique + Tonneau	Yes
8	Crushing - Destemming	No	Inox	Yes
9	Crushing - Destemming	Yes	Inox	Yes
10	Destemming - Crushing	No	Inox	Yes
11	Destemming - Crushing	Yes	Inox + Concrete	Yes
12	Destemming	Yes	Inox	Yes
13	Crushing - Destemming	Yes	Inox	No
14	Destemming - Crushing	No	Inox	Yes
15	Destemming - Crushing	Yes	Inox	Yes
16	Destemming	Yes	Inox	Yes
17	Destemming - Crushing	Yes	Inox	Yes
18	Crushing - Destemming	Yes	Inox	Yes
19	Destemming - Crushing	Yes	Inox	Yes
20	Crushing - Destemming	Yes	Inox	Yes
21	Crushing - Destemming	Yes	Inox	Yes
22	Destemming - Crushing	Yes	Inox	Yes

5.2.6. Malolactic fermentation – Tank

18 samples out of 22 carry out the malolactic fermentation in stainless steel tank. The company of the sample number 7 has chosen the barriques to carry out the second fermentation, since, at the time of the interview, the producer explained that his Nebbiolo appears more round and balanced compared to the malolactic fermentation in stainless steel. The company number 3 opts instead to use a barrel of larger size, the tonneau, so as not to have an inordinate influence of the hints of wood in the wine. Samples 15 and 16 carried out malolactic fermentation both in stainless steel tanks and in concrete tanks, to then be assembled at the end of the same.

5.2.7. Malolactic fermentation – Inoculum

Samples 9, 15, 16 and 19 decided to inoculate tanks with selected lactic bacteria, to avoid the development of other bacteria and unpleasant secondary products. The remaining 18 samples start the malolactic fermentation independently and naturally.

5.2.8. Malolactic fermentation – Heat

We know that malolactic fermentation is facilitated by higher temperatures than those present after alcoholic fermentation, since lactic bacteria are grouped among the thermophilic species; therefore the optimal temperature for their development is between 25 and 45 °C (Ribéreau-Gayon et al., 2005).

Most of the producers, in this case 14 out of the total of 22 samples, choose to heat the cellar environment or the tanks themselves in order to induce malolactic fermentation and make it happen immediately after alcoholic fermentation, so as to avoid the development of microorganisms which could give rise to unpleasant secondary compounds (Ribereau-Gayon et al. 2005).

Table 8 - Results of company survey form about malolactic fermentation, inoculation, heat

N° SAMPLE	MALOLACTIC FERMENTATION	INOCULUM MLF	HEAT
1	Inox	No	heat
2	Inox	No	
3	Tonneau	No	heat
4	Inox	No	heat
5	Inox	No	heat
6	Inox	No	heat
7	Barrique	No	
8	Inox	No	heat
9	Inox	Yes	heat
10	Inox	No	heat
11	Inox	No	heat
12	Inox	No	
13	Inox	No	
14	Inox	No	
15	Inox + concrete	Yes	
16	Inox + concrete	Yes	
17	Inox	No	heat
18	Inox	No	heat
19	Inox	Yes	
20	Inox	No	heat
21	Inox	No	heat
22	Inox	No	heat

5.2.9. Fining and stabilization

Protein stabilization occurs after the addition of protein material, such as isinglass or gelatine, because it could lead to overcolling and instability; the turbidity is eliminated with the addition of bentonite (Riberau-Gayon et al., 2005). Protein stability occurs before tartaric stability, as potassium bitartrate crystals are wrapped in protective colloids (Ribereau-Gayon et al., 2005).

On red wines it is chosen to have a more limpid wine, to stabilize the coloring matter, a greater microbiological stability and above all to attenuate excessive astringency, to reduce vegetable flavors and metallic scents (Grazietti, 2016). 6 samples out of 22 were treated with bentonite, to prevent protein casse (Grazietti, 2016).

On the total of the 5 samples of these, in addition to being treated with bentonite for stabilization, they add the isinglass for the fining that gives great brilliance to the wines (Grazietti, 2016). The sample number 20, instead of using animal gelatines, opts for a combination of bentonite for stabilization with protein plants, derived from pea proteins. A study carried out by Professor Grazietti in 2016 shows how compared to the treatment with gelatin, the wine with pea proteins is softer, with a more vivid coloring, better tasting volume, cleaner and clearer both on the "nose" and to the "mouth".

Finally, 10 samples out of 22 decided not to implement any clarification or stabilization. As far as tartaric stabilization is concerned, the choices of the producers are varied. Most, 14 samples for accuracy, apply to the tank very low temperatures, between -2 and -4 ° C, thanks to the insulation systems, to induce the precipitation of tartrate salts. To speed up the stabilization process, cream of tartar can be added simultaneously with the application of "cold", as the producer of the sample number 10 has opted.

7 samples out of the total do not implement particular precautions and wait for the tartaric stabilization to take place naturally.

5.2.10. Aging

The range of aging Nebbioli analyzed is very varied. Refinement is a decision that must be taken with caution and attention, since the evolution of wine during this period of time is impressive (Ribéreau-Gayon et al., 2005). In this project we can see how to switch from a non-aging Nebbiolo (like the number 2) to another that produces 36 months in wood of different sizes (sample number 14). However, as mentioned during the literature review, Nebbiolo is a wine that lends itself admirably to aging and aging in the bottle. Its evolution brings unique tertiary aromas to each type of Nebbiolo (Ribéreau-Gayon et al., 2005).

5.2.11. Aging container

The aging containers in this study are varied. 12 samples out of 22 choose the barriques, as the only solution or combined with larger woods, concrete and stainless steel. The levels of toasting and passages are numerous: from new barriques, to second and third passages, up to exhausted barriques. 8 samples prefer the use of the tonneau, of bigger

size than the barrique, in order not to have an excessive impact of the taste that releases the wood. Samples 9 and 10 are aging in stainless steel and samples 15 and 16 use concrete. Only companies in samples 2 and 3 choose no aging.

The gustatory and olfactory perception of the totality of the samples will be very different, not only because they use containers of different materials, but also for the time of aging.

Table 9 – Results of company survey form about fining, months' aging, aging container

N° SAMPLE	FINING	AGING (MONTHS)	AGING CONTAINER
1	Bentonite	6	Barrique 235L
2		No	No
3	Bentonite + Isinglass	18	French Tonneau
4	Bentonite	8	No
5		12	New Barrique light toasted, (75% inox, 25% barrique)
6		from 6 to 12	French Barrique 2° passage, (30% barrique, 70% inox o fiberglass)
7		from 18 to 24	Barrique and tonneau
8	Bentonite	8	French Barrique (3° passage)
9		8	Inox
10		from 6 to 12	Inox
11	Bentonite + Isinglass	12	Wood from 15hL to 100hL
12	Bentonite	24	Barrique and tonneau
13	Bentonite + Isinglass	24	Tonneau 2-3 passage
14		36	Barrique 225, tonneau 500 until 20hl
15	Bentonite + Isinglass	12	Concrete
16	Bentonite + Isinglass	24	Concrete, wood 30, 15, 5 tons and barrique
17	Bentonite	12	Barrique, Tonneau
18	Bentonite	12	Tonneau 2-3 passage (70%)
19		from 4 to 6	Barrique exhausted
20	Bentonite + Plant Protein	6 stainless + 12 barrique	Inox, Barrique
21		12	Barrique 3° passage
22		18-24	Barrique and tonneau

5.3. Base analysis

Table 10 - Basic analysis of the wines (1)

Sample	Year	Alcol % vol.	Residual sugars g/L	Total acidity g/L	Volatile acidity g/L	pH
1	2018	13.86	1.7	5.23	0.68	3.62
2	2018	12.86	2	7.34	0.39	3.22
3	2018	13.71	2.1	6.01	0.73	3.48
4	2018	13.93	3.5	6.44	1.04	3.49
5	2017	13.89	2	5.02	0.66	3.72
6	2017	14.19	1.1	4.87	0.65	3.57
7	2017	13.94	2	6.51	0.53	3.34
8	2017	13.3	2	6.13	0.96	3.49
9	2017	13.03	2.2	5.14	0.7	3.6
10	2017	12.36	2.8	5.77	0.73	3.47
11	2017	13.35	1.5	6.18	0.85	3.51
12	2016	14.36	1.9	5.87	1.03	3.58
13	2016	13.38	2.1	6.36	0.76	3.31
14	2016	14.01	1.4	6.15	0.85	3.48
15	2016	14.08	2.4	5.45	0.85	3.54
16	2016	13.05	2.3	5.55	0.86	3.57
17	2016	12.98	1.7	5.38	0.43	3.44
18	2016	13.24	2	6.05	0.87	3.49
19	2016	13.51	1.3	5.55	0.82	3.6
20	2016	13.26	1.5	5.98	0.86	3.38
21	2016	12.68	1.6	5.43	0.69	3.48
22	2015	13.71	2.4	5.48	0.7	3.56

Table 11 - Basic analysis of the wines (2)

Sample	Malic acid g/L	Lactic acid g/L	Tartaric acid g/L	Total dry extract g/L	Glycerol g/L	Ashes g/L
1	0	2.09	1.04	26.7	11	2.82
2	1.48	0.41	2.37	27.6	10.3	2.11
3	0	1.88	1.81	28.8	10.5	2.74
4	0.83	1.02	1.33	29.2	11.6	2.34
5	0	2.18	0.9	30.9	11.9	3.25
6	0	2.05	1.06	26.5	11.2	2.66
7	0.51	1.13	1.76	27.7	10.6	2.35
8	0.52	1.17	1.36	27.6	10.8	2.43
9	0	1.97	1.71	28.9	9.5	3.25
10	0	1.95	1.77	27.5	9	2.76
11	0	2.78	1.76	29	9.7	3.19
12	0	1.94	1.49	30.7	10.1	3.19
13	0	2.06	1.81	27.5	9.8	2.42
14	0	2.48	1.2	28.6	10.9	2.9
15	0	1.76	1.32	28.9	9.7	3.08
16	0	2.08	1.4	27.8	9.3	3.03
17	0	1.43	1.81	27.2	9.6	2.79
18	0	2.18	1.3	27.3	9.4	2.9
19	0	2.83	1.45	28.2	9.8	3.08
20	0	1.87	1.6	25.4	9.9	2.15
21	0	2.25	1.22	24.1	8.6	2.72
22	0	2.12	1.02	29	11.2	2.99

5.3.1. Residual sugar

The determination of residual sugars in musts is the first analysis that is carried out during vinification, as it allows to verify the correctness of alcoholic fermentation. The potential alcohol content is generally monitored with a mostimeter. In wines it needs to calculate the overall alcohol content, which must be included in the legal specifications, but above all to check that there are no subsequent technological problems. The amount of sugar that remains in the wine after alcoholic fermentation will have an impact on the evolution / aging and quality of the finished product. For example, malolactic fermentation must take place on wines considered dry, because the attack on sugars by lactic bacteria is dangerous; these actions, if they occur in an important way, can cause alterations, called "diseases", because of their microbiological nature. A wine is considered "dry" when the sugar content is less than 2 g/L, since up to this value the sugar residue is not detectable to taste (Ribereau-Gayon et al., 2005).

5.3.2. Total acidity

The sum of the indissociated and dissociated acids is represented by the total acidity, which represents the acidity of titration. Total acidity includes fixed acids (tartaric, malic, succinic, lactic, citric) and volatile acids. As a rule, the value of a red wine with total acidity ranges from 4.5 to 7 g/L of tartaric acid.

As for the samples, they are in the parameters listed above, showing a minimum of 4.87 g/L for the sample number 6 and a maximum of 7.34 g/L of the number 2; these values fall within the average of the total acidity of Nebbioli produced in other areas (Gerbi et al, 2006).

5.3.3. Volatile acidity

Acetic acid is the main acid to define volatile acidity (of which it is about 95%). It is a secondary product of alcoholic fermentation and is present in all wines: the contents due to alcoholic fermentation are small (0.2 g/L) if this has a normal course, also depending on the type of yeast strain that reacts and of the type of vinification: generally, the content of acetic acid that is formed is higher in red vinification rather than in white vinification (Ribereau-Gayon et al., 2005). With a correct malolactic fermentation the acetic acid content can reach 0,30 - 0,45 g/L. Acetic acid can also come from unhealthy grapes, such as those affected by acid rot and their winemaking causes an inoculation of acetic bacteria in wine.

The acetic acid content can also increase in the stages following fermentation: these increases are attributable to the action of microorganisms, essentially bacteria: the control of acetic acid and volatile acidity therefore becomes a fundamental factor in the monitoring of winemaking processes, because it highlights possible states of alteration in progress (Ribereau-Gayon et al., 2005).

In red wines, if they undergo malolactic fermentation, the content of acetic acid increases (and, therefore, volatile acidity) because some lactic bacteria that govern malo-lactic fermentation have an heterofermentative action. Furthermore, from the unfermentable sugars, from the yeasts and from the citric acid there is a further formation of acetic acid. It becomes important that there are no sudden increases in the acetic acid content, a symptom of an unwanted microbial activity. Another process that needs to be emphasized to avoid excessive increases in volatile acidity is the aging in wood and the related oxygenation. There is also an increase in the acetic acid content also in the aging in wood, especially in small container. The "micro-oxygenation" that takes place during this process, can facilitate, if it does not take place in adequate hygienic conditions, the development of acetic bacteria, even at the level of barriques. The parts of the wooden insertions in direct contact with the wine, such as the insertions of the staves and the bung-hole, with the passage of oxygen create the conditions for the proliferation of acetic bacteria, with the risk that the volatile acidity becomes excessive.

The volatile acidity content of wines is regulated in Reg. CE 606/2009 Allegato I C., with a maximum legal limit of 1.2 g/L as acetic acid in red wines. The volatile acidity becomes a defect when it exceeds 0.8 g/L and is called "acescenza". A fermentation stop can be dangerous, because at the end of alcoholic fermentation it could be 0.6-0.7 g/L of volatile acidity, which makes malolactic fermentation dangerous.

All 22 samples have a volatile acidity below the legal limit. Samples 4 and 12 have strong volatile acidities, both above 1 g/L. One of the causes of this increase in volatile acidity, for sample number 12, is the effect of maceration, being 90 days.

5.3.4. pH

During the various stages of vinification and storage, the pH changes; the pH of musts varies from 2.8 to 3.6, while that of wines is between 2.8 and 3.8. This parameter is of primary importance during wine making and the success of a wine: the hue and color intensity of red wines depends on their pH; higher is the pH, less colour we have in the wine (Ribereau-Gayon et al., 2005). The chemical reactions that microorganisms perform

in wine are pH dependent; only when it is equal to or greater than 3.5 the malolactic fermentation begins.

Also in this case, the results fall within the generic standards of wine pH. We can also say that sample 2 has the lowest value (3.22), since malolactic fermentation was still in progress at the time of sampling. As a result, the pH in this case will increase at the end of the second fermentation. However, the Nebbiolo of the areas considered, shows marked acidity and with not high pH values (Zeppa et al., 2008).

5.3.5. Malic acid

The variety and the place of origin also greatly influence the concentration of malic acid; in fact, in the must, before malolactic fermentation, the average analytical concentrations of malic acid are 4-8 g/L in cold areas, 1-2 g/L in hot areas. Its concentration is reduced mainly due to malolactic fermentation, bringing the quantity to 0 - 5 g/L.

18 samples out of 22 have completed malolactic fermentation, since the concentration of the aforementioned acid is 0 g/L; only the samples number 2, 4, 7 and 8 have more or less high concentrations. It is understandable that the two samples of 2018 (n° 2 and 4) have the highest levels of malic acid, since at the time of sampling, the wines were still in the tank and both were not "finished" wines and ready for aging and subsequent bottling.

In the case of the numbers 7 and 8, it is evident that the malolactic fermentation has not come to an end, not by the will of the producer but by chance. This residue of malic acid contributes to the total acidity of the two Nebbiolos, which on tasting were more sour and less enveloping, but above all problems can be encountered from the technological point of view with refermentations in the bottle. The product, in general, is less stable microbiologically (Ribéreau-Gayon et al., 2005).

5.3.6. Lactic acid

It is important to perform an analysis on the quantities of lactic acid present in the wine, as for the malic acid, to understand if the malo-lactic fermentation has been completed or not. The concentration can vary between 0 and 5 gr/L and its origin is due to the process of chemical transformation, secondary fermentation, which leads from malic acid to lactic acid. The lowest concentrations of malic acid are the same that had a high content of malic acid, then the samples 2, 4, 7, and 8. The lowest value was found in the sample number 2, with 0.41 g/L of malic acid.

5.4. Phenolic and colour analysis

Performing the analysis during winemaking, the polyphenolic framework and the coloring matter of a wine, especially on Nebbiolo, can provide a forecast on the evolution over time of the various compounds and how they will increase or decrease during aging and further bottle aging. Making analysis on bottled wine, having an image of the relationship of proanthocyanidins, vanillin-reactive flavanols, total anthocyanins and keeping a historic over the years allows us to monitor progress and act in case of need (Rolle, Personal communication, lessons of Technological Innovation in Oenology. Academic year 2017/2018). It is important to better manage storage in the bottle and choose the best closure for a wine with a very high evolutionary potential in glass.

Furthermore, thanks to studies previously carried out by Cravero, Di Stefano, Borsa and Gentilini in the 1990s, we have not only a quantitative but also a qualitative knowledge of the phenols of the Nebbiolo grape variety and how we can best manage the extraction of the color from the skins during fermentation.

5.4.1. Total polyphenols

The total polyphenols of these 22 samples were analyzed using the Folin-Ciocalteu index. The values are very variable: the minimum is the sample number 21 with 1799 mg/L (+)-catechin up to a maximum of 3029 mg/L of the sample number 4.

Theoretically, the value of the total polyphenols, with the passage of time and with the progress of aging of a wine, decreases. This is due to the oxidation of polyphenols, their polymerization and precipitation (Ribereau-Gayon et al., 2005).

The sample number 1, despite being the 2018 vintage, has among the lowest values of the samples analyzed (1851 mg/L (+)-catechin). It is assumed that this variability is given above all by the vintage, also by the different percentage of Nebbiolo inside the samples, by the harvest, by the condition of the grapes, by the vinification methods and above all by the period of aging.

The estimate of total polyphenols, made with Folin-Ciocalteu, has the limitation of taking into consideration other substances not typically polyphenols, and for practical purposes it is a number that does not give us the division between tannins and anthocyanins. Two wines may have the same value, but one being rich in tannins and poor in color and the other vice versa.

Table 12 - Total polyphenols of the wines (intensity of colour grows with the concentration)

SAMPLE	Total polyphenols (mg/L (+)-catechina)
1	1851
2	2288
3	2475
4	3029
5	1931
6	2468
7	2773
8	2017
9	2492
10	1872
11	2284
12	2749
13	2534
14	2440
15	2669
16	2229
17	2870
18	2121
19	2395
20	2222
21	1799
22	2405

5.4.2. Total flavonoids

When we calculate the total flavonoids of a wine, we have the expression of polymeric tannins (proanthocyanidins) and oligomers (vanilla-flavored flavanols); one has a general idea of the tannic content of wine and how to manage it, especially in winemaking, with regard to when racking to avoid extractions that are too high or too short.

Also in this analysis, the extreme values have a range of more than 1300 mg / L (+) - catechin. The lowest is found for the sample number 21 (as in the case of total polyphenols), and is 1232 mg / L, the highest belongs to the number 12, a Carema of 2017, of 2583 mg / L.

Just this last value, matches that of a Carema of the 2000s (Zeppa et al., 2008), and we can see that the overall results, compared to studies of Nebbioli coming from different areas of production, fall within a normal range (Gerbi et al., 2006).

Table 13- Total flavonoids of the wines (intensity of colour grows with the concentration)

SAMPLE	Total Flavonoids
	mg/L (+)-catechine
1	1714
2	1735
3	2105
4	2414
5	1545
6	2097
7	2357
8	1376
9	1796
10	1269
11	1594
12	2583
13	2035
14	1623
15	2171
16	1446
17	2497
18	1833
19	1870
20	1838
21	1232
22	1726

Along the evolutionary arc of wine, total flavonoids tend to decrease and at the same time increase proanthocyanidins, due to polymerization and precipitation reactions of tannins with tartrate salts and coloring matter.

5.4.3. Total and monomer anthocyanins

As explained in the literature review, the total anthocyanins are represented by the sum of free monomers and those linked with tannins. In the grapes the anthocyanins are almost all monomers, during the vinification process a portion of them will bind with the tannins, one part will remain free and another will be lost. Consequently the anthocyanin-tannin bond must be favored as much as possible, thanks to techniques such as micro-oxygenation and aging through the action of oxygen and consequently of acetaldehyde and its ethyl bridges, they increase the share of monomer anthocyanins that bind, making the compound more stable.

When I make an analysis on the color of a wine I determine the total anthocyanins, an important fact but it is necessary to understand how much the monomer anthocyanins contribute to the total; consequently it is also necessary to analyze the free ones.

If the total anthocyanins and monomers decreased in parallel it would indicate a non-ability of the oenologist to safeguard the monomer anthocyanins. Examples are the samples number 6 of 2017, 100% Nebbiolo, which has 58 mg / L of total anthocyanins (really low quantity for a wine of not even 2 years) and 8 mg / L of monomer anthocyanins and the number 14 of 2016, Nebbiolo in purity, with 45 mg / L of total anthocyanins and 4 mg / L of monomer anthocyanins.

The oenologist must therefore decide how and when to use the techniques available to him such as micro-oxygenation, sulfitation to preserve oxidation, and aging in wood. All these methods are intended to transform the largest possible amount of monomers into total anthocyanins. So analytically you can see how they impact the techniques adopted on both components. The bottling depends on the type of wine you want to produce; if we talk about wines of long conservation (eg Barbaresco, Barolo), the analysis that we will see do not have much meaning because time leads to a natural evolution of the monomer anthocyanins in total anthocyanins.

Speaking of the totality of the samples, as regards the total anthocyanins, the analysis carried out have a minimum of 45 mg/L (sample number 14, 100% Nebbiolo, 2016) and a maximum of 175 mg/ L (sample number 4, 100% Nebbiolo, 2018). Comparing the data with two previously cited works (Zeppa et al., 2008, Gerbi et al., 2006), in both cases the wines (Carema and Barbaresco) were from 3 to 6 years of age when the study was implemented and the total anthocyanins range showed between 98 mg/L and 102 mg/L. To confirm this, a study by Mannini et al in 2006, shows values, both in the Langhe area and in Donnas, an area also interested in this thesis, higher than the average mentioned above.

Let's analyse the monomer anthocyanins: the minimum value is represented by the sample number 14, year 2016, 100% Nebbiolo with 4 mg/L and a maximum of 59 mg/L for the sample 5, year 2017, also in purity. The average in this case is around 23 mg/L malvidin-3-glucoside cl and more than half of the samples are below the sample mean. This is confirmed by the study on the Barbaresco of Gerbi et al, in 2006, where the 4 Barbaresco between the years 1998 and 2001, have values respectively of 30 mg/L, 24 mg/L, 24 mg/L and 19 mg/L. Remember that in the amount of anthocyanins both total and monomers is influenced by the altitude, the soil, the clone and the management of the vineyard as well as the method of vinification and aging (Mattivi et al., 2006).

Table 14 - Total and monomer anthocyanins of the wines (intensity of colour grows with the concentration)

SAMPLE	Total anthocyanins	Monomer anthocyanins
	mg/L malvidine-3-glucoside cl	mg/L malvidine-3-glucoside cl
1	102	41
2	97	44
3	137	54
4	175	22
5	141	59
6	58	8
7	97	27
8	77	10
9	106	35
10	115	43
11	61	14
12	66	13
13	68	6
14	45	4
15	88	7
16	108	23
17	138	19
18	105	25
19	70	17
20	51	5
21	88	20
22	48	10

5.4.4. Proanthocyanidins

The monomer forms of flavan-3-ols are present in grapes in limited quantities: the flavan-3-ols are found above all in polymer form and their polymers are called proanthocyanidins. Proanthocyanidins are also referred to as condensed tannins: the term tannin does not indicate a class of chemical compounds, but rather a set of polyphenolic substances that have the ability to originate stable combinations with proteins and other vegetable polymers.

Comparing the values of this thesis with those of the works cited above (Zeppa et al., 2008, Gerbi et al., 2006), it can be seen that they fall for the most part within the range of acceptability. A value only, it differs from the general trend of Nebbioli in this study: the sample number 10, 100% Nebbiolo, 2017 has the lowest value of the 22 samples, that is 1832 mg / L cyanidin chloride. This value is not found in any of the previous studies carried out on Nebbiolo wines. The maximum value detected is 3963 mg / L cyanidin chloride, since it deviates more than 1200 mg / L from the sample mean.

Table 15 - Proanthocyanidins of the wines (intensity of colour grows with the concentration)

SAMPLE	Proanthocyanidins mg/L cianidine clorure
1	2421
2	2290
3	3253
4	3383
5	2346
6	2860
7	3337
8	2299
9	2795
10	1832
11	2365
12	3187
13	3168
14	3262
15	3309
16	2795
17	3963
18	2439
19	2823
20	2692
21	2140
22	2589

5.4.5. Vanillin reactive flavanols

Vanillin-reactive flavanes are compounds formed from polymerized molecules of 4-6 units of flavan-3-ols monomers. Proanthocyanidins 6-7-8 and up. The estimate of total tannins is often overestimated as there is a small part of compounds of both overlapping classes (Di Stefano et al., 1989, Di Stefano, Cravero, 1991). Being of low molar mass they give bitter sensory characteristics, which if they are not well balanced, may be unpleasant to the palate (Robichaud, et al., 1990).

Only 3 samples are below the range found also in other studies. Sample 8 (2017, 100% Nebbiolo), has vanilla flavanols 606 mg / L, expressed in (+) - catechin, sample 10 (2017, 100% Nebbiolo) 763 mg / L and finally number 21, with 694 mg / L. the resulting maximum value is 1785 mg / L (+) - catechin.

Table 16 - Vanillin reactive flavanols of the wines (intensity of colour grows with the concentration)

SAMPLE	FRV
	mg/L (+)-catechine
1	1222
2	1200
3	1514
4	1785
5	1062
6	1222
7	1722
8	606
9	1411
10	763
11	1159
12	1785
13	1461
14	1417
15	1492
16	1552
17	1580
18	1480
19	1407
20	980
21	694
22	1150

5.4.6. The colour

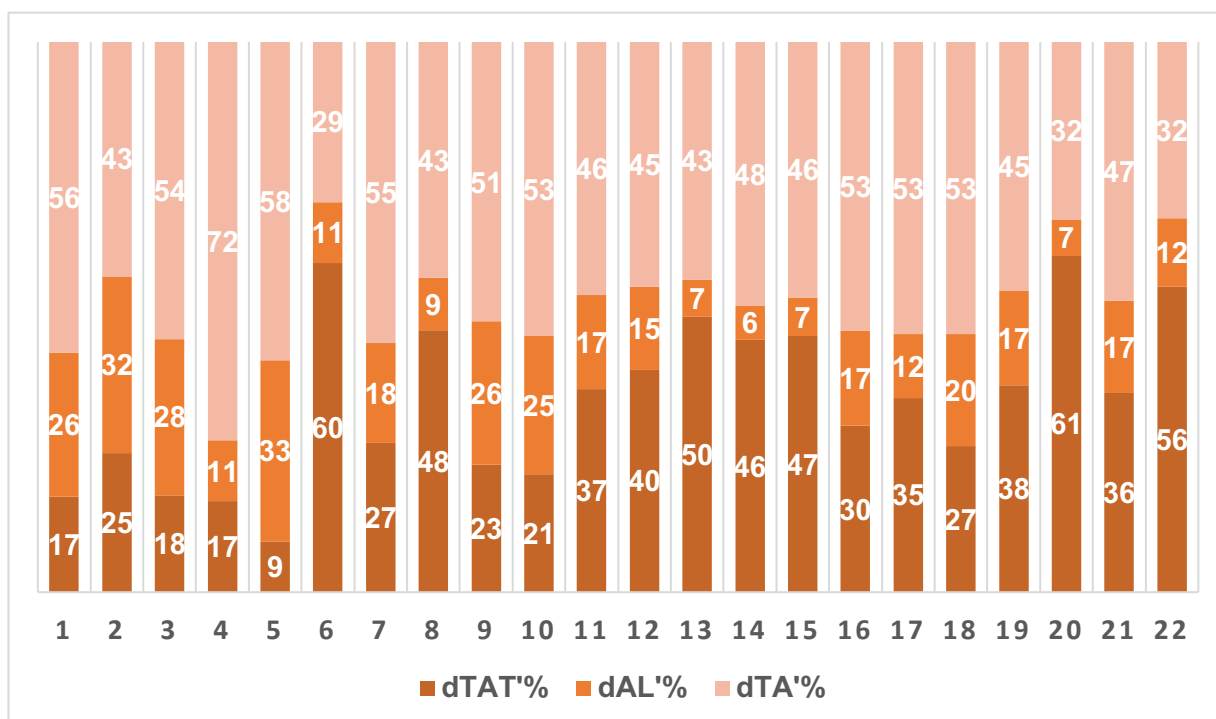
5.4.6.1. Fractionation and wine color indexes at pH 0 (%)

It is of fundamental importance to keep the coloring pigments, stabilize them and not risk losing them. It is necessary to know the content of monomer anthocyanins and how sensitive they are to external agents. To study how anthocyanins are combined, we can carry out an analysis that leads to color indexes by fractionation, but instead of pH of wine, at pH 0, where all pigments ionize.

These indexes are dAL%, dTA% and dTAT% which represent what happens between the total and monomeric component of the wines during the process. The dAL describes the percentage of absorbance at 520 nm (that of the red color) which is linked to the monomer anthocyanins. The dTA represents the anthocyanins already bound, or that are binding to the tannic component but which can still be bleached with the addition of SO₂. The dTAT is the part of anthocyanins consolidated with tannins and therefore stable. Thanks to the course of studies held by Professor Rolle, during the first year of master of viticultural and enological sciences, we talked about different situations with different amounts of these indexes. A wine with 3 years of aging will have an average of 5% of monomer anthocyanins

(dAL%), 10% of pigments sensitive to SO₂ dTA% and 85% of dTAT% (Rolle, personal communication, lessons of Technological Innovation in Oenology , Academic Year 2017/2018).

In this thesis we analyzed wines with a maximum aging of 3 years (2015) up to some samples that had been vinified 6 months before; consequently the values will be different comparing them with previously conducted studies.



dTAT% = **Pigments NOT sensible to SO₂**

dAL% = **Monomers anthocyanidins**

dAT% = **Sensible pigments to SO₂**

Figure 4 - Fractionation and color indexes of the wines at ph 0 (%)

As we can see from figure 4, the general picture is diversified. We can affirm that the first 4 samples (n ° 1-4) being of the 2018 vintage, have a reduced quantity of dTAT%, due to a time factor; the wine has been maturing for a few months and consequently the stabilization of the anthocyanins through the links with the tannins has yet to be completed. We must therefore pay close attention to the anthocyanins monomers, but above all to the dTA% (with the highest percentage), since I add to the wines of sulfur dioxide, I will go to bleach the pigments that are not fixed yet.

Moving on to the samples of the year 2017, from the number 5 to the number 11, we note that the percentages of the three indexes of the samples 7,8,9,10,11, have a linear trend, always recommending an interest in the high percentages of the pigments sensitive to SO₂.

Contrasting situations, are those of the samples number 5 and number 6. The sample number 5, despite being the year 2017, therefore a young age for the "long-lived" Nebbiolo, has a very light color, as evidenced also by the quantity of pigments stable and not sensitive to SO₂ (9% dTAT). Given even more alarming is the percentage of pigments sensitive to sulfur: 58%. At the time of sampling, the sample was extracted from the barriques so it is not yet finished. This means that at the time of a future bottling and addition of potassium metabisulfite to protect the wine, other pigments will be removed from the mass.



Figure 5 - Picture of sample No. 5 during the analysis

The opposite condition is the one presented with sample number 6, which presents a darker color at the time of analysis. Also from 2017 and taken from aging vats, it has a dTAT% of 60; this means that the pigments have been mostly stabilized and at the time of the addition of an antioxidant, they will not be subtracted.



Figure 6 - Picture of sample No. 6 during the analysis

As for the 2016 samples, between the number 12 and the number 21, we note that the percentages of pigments not sensitive to SO_2 have increased, given the greater aging time, however the percentage of sensitive pigments remains high.

Finally, sample number 22, of 2015, is among the top 3 with the highest percentage of pigments not susceptible to bleaching. The samples that are more stable to the effect of sulfur are all "Canavese Nebbiolo DOC".

In this work, when we talk about color and its stabilization, we must always keep in mind the many variables that come into play: first of all in the viticultural part the exposure, the altitude, the form of farming and the land. As for the oenological part, the situation is even more variable. For example, if it is a Nebbiolo vinified alone or blended with varieties with a more pronounced anthocyanin profile, such as Chatus, Croatina and Neretto di Bairo (Gerbi et al., 2005), the winemaker, maceration, pressing, fermentation and additives that can go to remove color from the mass (Ribereau-Gayon et al., 2005).

In a very young wine to accelerate the reduction of free and SO_2 sensitive pigments, I can opt for micro-oxygenation, before or after malo-lactic fermentation, to increase the speed of tannin-anthocyanin union (Rolle, Personal Communication, Innovation lessons Technology in Oenology, Academic Year 2017/2018).

5.4.6.2. Colour intensity, colour tone and cielab colorimetric indexes

As previously mentioned the color of a wine is a fundamental parameter for the visual and qualitative evaluation by the consumer. The parameters analyzed will be compared with more studies carried out on both Carema DOC, Barolo DOCG, Barbaresco DOCG, Nebbiolo d'Alba DOC, Roero DOC, Nebbiolo "young" and aged Nebbiolo (Zeppa et al., 2008, Gerbi et al., 2006, Cagnasso et al., 2007, Cagnasso et al., 2007).

The sample number 5 is characterized by the lowest value of coloring intensity (2.43 PO 10mm), with higher brightness value, therefore appears clearer than all. The maximum recorded value is 7.12, with the sample number 17, Nebbiolo in purity, 2016; this value is very similar to that found in the study by Gerbi and colleagues in 2006, on a Barbaresco DOCG from 1999 (it was 7.13). The comparison between the 4 samples of Carema DOC 2017/2016 (n ° 11-14) and that of the Zeppa study of 2008, where the coloring intensity was 5.53, is negative for the Carema of this study: 3 samples on 4, respectively the numbers 11, 12 and 14, are lower than that of 2008. Confirming this result is the content of total anthocyanins, between 68 mg/L and 45 mg/L.

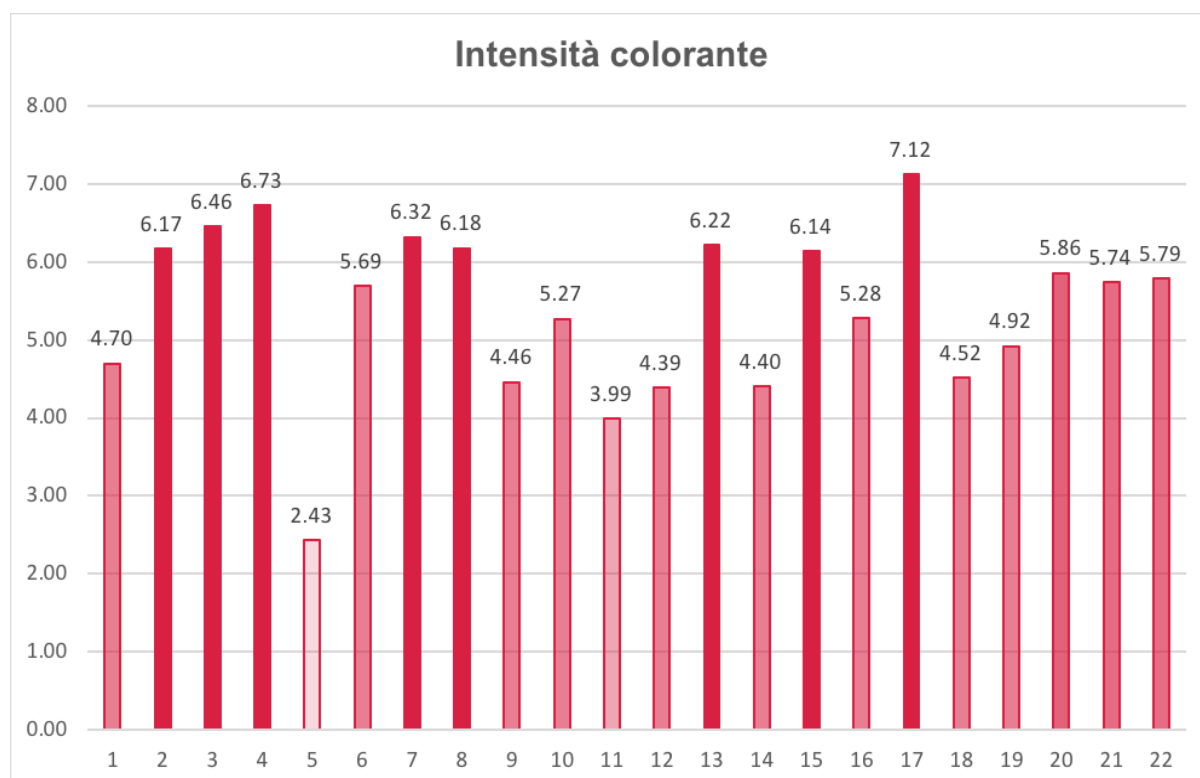


Figure 7 - Color intensity of the wines

As previously expressed, the coloring shade is given by the ratio between absorbance at 420nm and 520nm and the higher this value, the more a wine will tend to orange tones

(Glories, 1984). Analysing Figure 8 below, the first 4 samples of 2018, all 100% Nebbiolo, have the lowest values of the sample population, characterized by red color, being the youngest and the aging started recently. 3 samples of Carema out of 4 (11,12,14) have coloring shades that start at 1.20; this is a high value and the wine tends to orange/garnet (Zeppa et al., 2008). These values can be justified by the disciplinary, which determines 24 months of aging the oak or chestnut wood; aging in wood brings some modifications to the colored pigments, forming vitisins A and B, from the typical orange color (Ribereau-Gayon et al., 2005).

This also applies to the subsequent samples, all with high values of coloring tone. Nebbiolo, being a variety with few synthesized anthocyanins, if they are not extracted and protected in the best way, they will oxidize and lose the typical red color of young Nebbioli; above all the innate nature tending to aging that characterizes this cultivar.

Nebbiolo is a variety, as mentioned previously rich in tannins that are detrimental to anthocyanins. The tannins, so numerous, tend to combine with each other (T-T), causing browning of the color of the wine and combining with the anthocyanins directly (T-A+), determining a red-orange color (Fulcrand et al., 2004) . The reactions mediated by the ethyl bridges between anthocyanins and tannins (T-et-A+) give rise to the red-purple color, which is not found particularly in the samples under examination. These last compounds, are not completely stable and with the passage of time react with the piranoantocyanins, of orange color to form the final compound called "flavanil-piranoantociani" (Cagnasso et al., 2007).

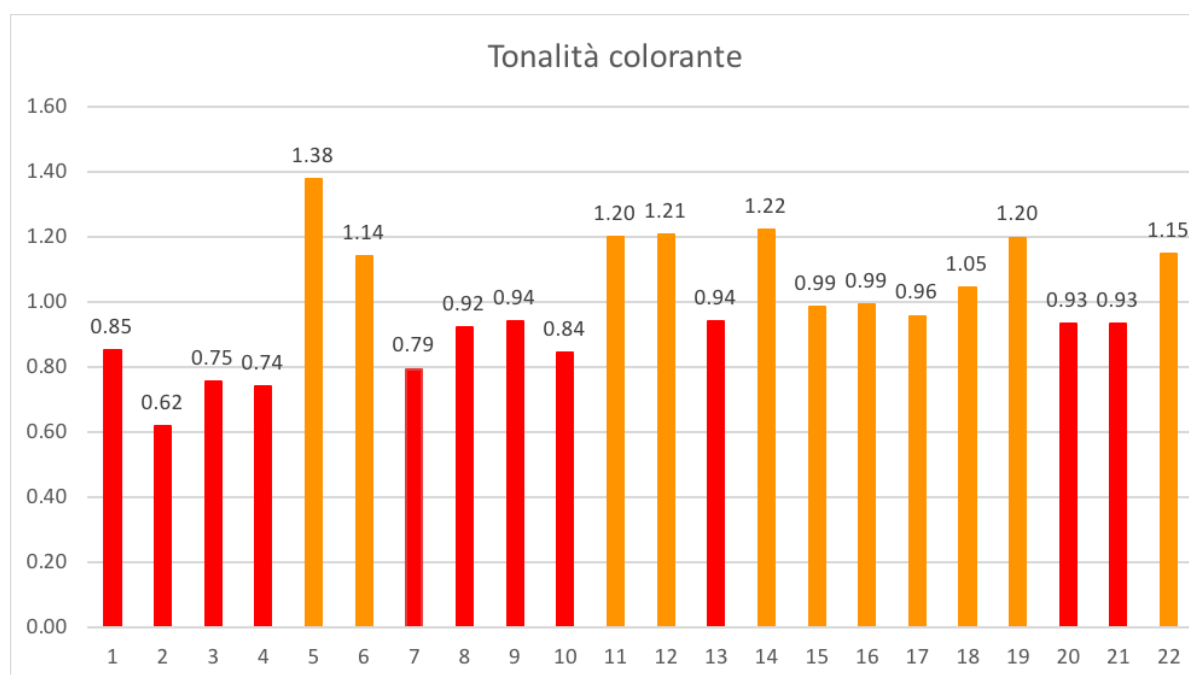


Figure 8 - Coloring tone of the wines

The CIELab indexes indicate the brightness of a wine, if it tends more towards green or red, and to yellow or blue. L^* stands for brightness and goes from 0 to 100, the higher this value, the clearer the Nebbiolo taken into analysis will be. As can be seen from the data collected, the sample number 5 has a brightness of 57.86; this data confirms the color at the time of opening for analysis. Moreover, it has the lowest value of a^* (39.82), which the lower it is, the more it will tend to green. The brightness of the other samples, excluding some sporadic cases (n ° 11, 12, 14, 18), are in line with the other studies conducted on various types of Nebbiolo (Zeppa et al., 2008, Gerbi et al., 2006, Cagnasso et al., 2007, Cagnasso et al., 2007). As for the red value, a^* , the first 4 samples and the number 7, are those with the highest results, between 56.49 and 57.78; they are characterized by a more vivid red color. Also for this datum, the rest of the samples show an average trend in line with the previous works (Zeppa et al., 2008, Gerbi et al., 2006, Cagnasso et al., 2007, Cagnasso et al., 2007). The last value b^* , describes two specific colors, $-b^*$ corresponds to blue and $+b^*$ to yellow. Figure 18 shows, as the sample number 14, Carema DOC, 2016, has the highest value of all the samples (53.40), very similar to the value recorded in the study by Zeppa and colleagues in 2008 (55.87) . As a result, this sample is the most yellowish.

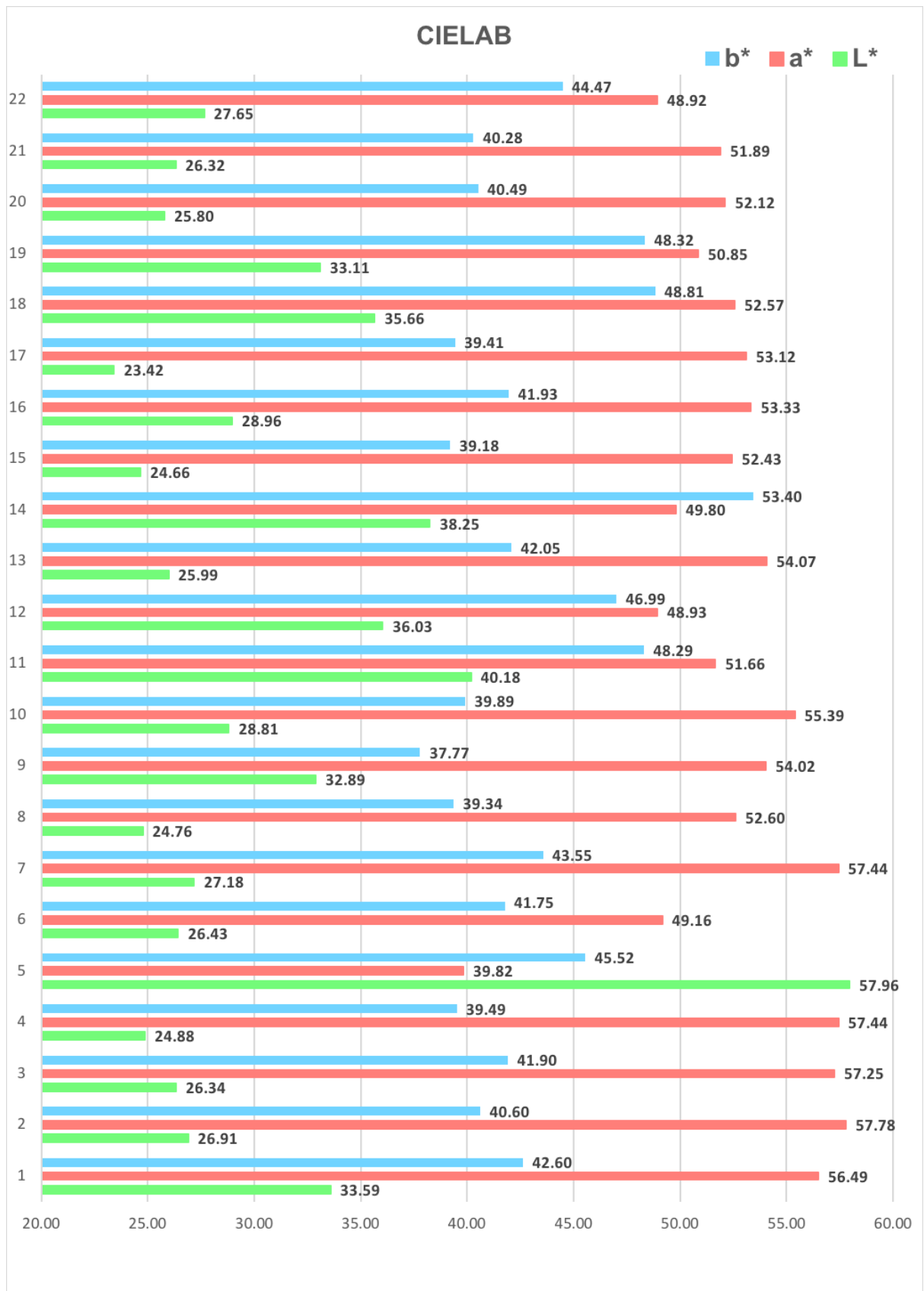


Figure 9 - Values of L*, a* and b* of the wines

5.4.6.3. Polymeric pigments analyzed with bovine serum albumin (BSA) and bleaching by bisolphite

After analyzing the color composition of the 22 Nebbiolo samples, using total anthocyanins (mg/L malvidin 3-O-glucoside chloride), anthocyanins monomers (mg/L malvidin 3-O-glucoside chloride), color intensity, coloring tone, CIELab indexes, fractionation and color indexes of wines at pH 0, expressed as a percentage, to detect monomer pigments, sensitive and not to SO₂, a specific analysis was carried out, studied by Harbertson and colleagues in 2003, to measure polymer pigments. Starting from previously implemented studies, the polymeric pigments consist of anthocyanins linked to tannins, these can be differentiated into two groups: those that do not precipitate with proteins, short-chain polymeric pigments (short polymeric pigments, SPP) and those that precipitate with proteins, given their size, called long chain polymeric pigments (long polymeric pigments, LPP) (Harbertson et al., 2003).

In fact, the analysis exploits the ability of larger tannins, or oligomers and polymers tannins (units > 3) to be precipitated by proteins (Hagerman et al., 1978; Ma et al., 2016).

The analysis carried out in the laboratory combines the bleaching of bisulfite with the precipitation of a particular protein, bovine serum albumin (BSA), differentiating the monomer anthocyanins and polymeric pigments; divided into the groups described above (SPP, LPP). The latter go to benefit the stability of the color of the wine, since the more SPP and LPP form and will be formed, more color will be fixed (Villamor et al., 2009).

Many studies show the importance of polymerization starting from the alcoholic fermentation, up to the aging in the bottle. In particular, Harbertson et al., (2003), showed that in the first stages of vinification more SPP are formed, whereas the formation of LPP is favored more during bottle aging (Villamor et al., 2009).

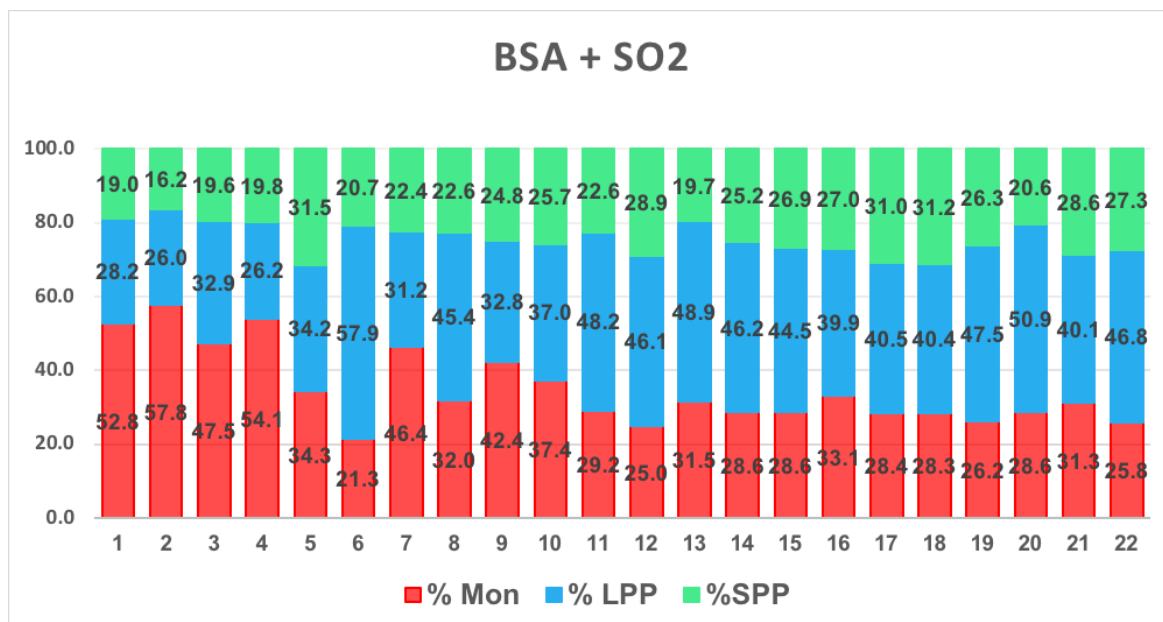


Figure 10 - Anthocyanidins monomers, LPP, SPP of the 22 samples

The figure above represents the situation of the 22 samples, after the analysis with the BSA and the bleaching with bisulfite.

As expected, the first 4 samples, all pure Nebbiolo and 2018, vinified for a few months relatively, have a higher content of monomer anthocyanins than the rest of the wines, on the contrary the SPPs have the lowest percentages among the samples.

Despite being very young wines, LPP values appear to be higher than short polymeric pigments. The aging in wood, which is small, like the barrique, which is larger, like the tonneau, is useful for the polymerization between anthocyanins and tannins; in fact the lowest value is found in sample number 2, which does not aged in wood.

The progression of the subsequent samples is generally linear both in the case in which they are still in aging, and whether they have already been bottled. An isolated case, as previously described in the fractionation analysis, is sample number 6, which shows the highest LPP value (57.9%). From the values we have obtained, both in the case of fractionation, and in this specific analysis, and as shown in the photo above, the sample number 6 appears to be the most stable in terms of coloring matter.

6. CONCLUSION

The present work, carried out in the year 2018/2019, was born as a consequence of an activity that the province of Turin carries out in the framework of an ALCOTRA project, called " Strada dei vigneti alpini", which involves the Valle d'Aosta Region, the Cervim, the

Institut Agricole Régional, the Municipalities of Carema and Pomaretto and other French administrations. The aims of the project are the enhancement of the wines for the tourism purposes, the vineyard landscapes of the areas involved and the producers themselves. The need to verify the state of art on production techniques in the vineyard and in the cellar was implicit. Therefore, this work gives an unfinished contribution both to the knowledge of the wines of the territories examined and from the quantitative point of view, since the companies that were not included in the study are numerous. Despite this, it is of great interest for the deepening of the behavior of Nebbiolo grapes in piedmont situations or in pedological situations different from the traditional ones of Langa and Roero.

Of the 22 wines analyzed, the Nebbiolo imprint, the position, the vineyard management, the climate and the altitude which profoundly affect the expression of the wine, are perfectly recognizable. Certainly also the vinification techniques and the equipment used need to be improved, in order to obtain an increase in the final quality of the product. The analysis done, show the knowledge of the subject still to be deepened and integrated by the producers, except for some sporadic cases. Colour, a fundamental point in the tasting of a wine, must be protected and taken into consideration as a strong point of the product. However, the results confirm that the Nebbiolo of these areas is expressed in a potentially interesting way also from the commercial point of view. Reconnecting to the initial purpose, namely that of identifying the possibility of consolidating the wine-making activity of the territory, the answer is undoubtedly positive.

In my personal opinion, we should give more voice to Nebbioli that are found outside very famous production areas of Barolo and Barbaresco, not only for the great strength that the cultivar releases in the studied areas, also because shoppers often buy the denomination and the area of origin, rather than the quality of the contents in the bottle.

7. ATTACHMENTS

SCHEDA PER IL RILIEVO AZIENDALE

DESCRIZIONE AZIENDA	
• Denominazione:
• Sede:
 Tel.....
• Appartenenza a Consorzio di Tutela :
• Tipologie di vini prodotti in azienda:

• Superficie a vigneto:
• Età e Altitudine vigneto:
• Pergola (Topia) o filare:
• Potatura:
• Portinnesto e barbatelle:
• Resa vigneto:
• Vitigni coltivati (%):
• Conduzione vigneto (i.e. Biologica, Convenzionale, Integrata, Biodinamica):
• Quantità dei vini prodotti:

• Provenienza, modalità di raccolta e trasporto delle uve:

• Analisi sulle uve alla vendemmia:

• Analisi di processo e sul vino:

• Risorse umane impiegate (lavoro familiare, personale esterno, tecnico enologo, consulente):

• Commercializzazione del vino (sfuso, imbottigliato -%):

Figure 11- Survey form, description of the company

BIBLIOGRAPHY

- Bakker, J., and C.F. Timberlake. (1997). Isolation, identification, and characterization of new color-stable anthocyanins occurring in some red wines. *J. Agric. Food Chem.* 45. 35-43.
- Benabdeljalil, C., V. Cheynier, H. Fulcrand, A. Hakiki, M. Mosaddak, and M. Moutounet. (2000). Mise en évidence de nouveaux pigments formés par réaction des anthocyanes avec des métabolites de levures. *Sci. Aliments* 20. 203-220.
- Berta P., Mainardi G. (1997). Storia regionale della vite e del vino in Italia: Piemonte. Collana accademica italiana vite e vino. Ed. Unione Italiana Vini, Milano.
- Bettiga L. (2003). Wine grape varieties in California. *Agriculture & Natural Resources*. 103.
- Bosso A., Guaita M., Ballario P. (2004). L'estrazione differita degli antociani abbinata alla tecnica del délestage nella vinificazione di uve Nebbiolo. *Rivista Viticola Enologica*. 57, 1/2, 29-49.
- Bosso A., Panero L., Guaita M., Petroziello M. (2007). La tecnica di macerazione prefermentativa a freddo con neve carbonica nella vinificazione in rosso di alcune cultivar italiane. *Atti XXX congresso internazionale OIV*.
- Brochet F. (2001). La dégustation. Étude des représentations des objets chimiques dans le champ de la connaissance. Prix Coup de Coeur 2001, Académie Amarin, Paris, F.
- Brochet F., Dubordieu D. (2001). Wine descriptive language supports cognitive specificity of the chemical senses. *Brain and Language* 77, 2. 187-196.
- Cagnasso E. (1997). Metodi oggettivi per la definizione del colore del vino. *Quad. Vitic. Enol. Univ. Torino*, 21, 129-152.
- Cagnasso E., Ummarino I., Di Stefano R. (2001). Estrazione dei polifenoli di uve 'Nebbiolo' da Barolo con sistemi diversi di vinificazione. *L'Enologo*. 37. 4. 61-80.
- Cagnasso E., Rolle L., Caudana A., Zeppa G., Gerbi V. (2007). Profilo cromatico di vini rossi piemontesi. *Atti Enoforum*. 91.
- Cagnasso E., Zeppa G., Rolle L., Caudana A., Gerbi V. (2007). Il colore dei vini derivati dal "Nebbiolo". 29. *Quaderni di scienze viticole ed enologiche*. 169-183.
- Cordero di Pamparato S. (1265-1300). Documenti per la storia del Piemonte.
- Cravero M.C., Di Stefano R. (1992). Composizione fenolica di alcune varietà di vite coltivate in Piemonte. *Vignevini*. 05. 47-5.
- Dalmasso G., Dell'Olio G., Corte A. (1962). Principali vitigni da vino coltivati in Italia. Ministero dell'Agricoltura e delle Foreste. Volume II.
- De' Crescenzi P. (1305). *Opus ruralium commodorum*. Libri XII.
- Di Stefano R., Cravero M.C., Gentilini N. (1989). Metodi per lo studio dei polifenoli dei vini. *L'Enotecnico*. 25. 83-89.

- Di Stefano R., Cravero M.C. (1991). The grape phenolic determination. *Riv. Vitic. Enol.* 2. 37-45.
- Di Stefano R., Borsa R., Gentilini N., (1994). Estrazione degli antociani dalle bucce dell'uva durante la fermentazione. *L'Enotecnico.* 05. 75-83.
- Versari A., Parpinello G.P., Galassi S. (2008). Metodi di analisi del colore dei vini rossi: antociani, pigmenti polimerici e copigmentazione. *Enotecnico* 5, 93–102.
- Di Stefano R., Bosso A., Panero L., Follis R. (2003). Nuove tecniche di vinificazione in rosso. *Vinidea.net*, rivista internet tecnica del vino. 1: 1-10.
- Fulcrand H., Atasanova V., Salas E., Cheynier V. (2004). The fate of anthocyanins in wine: are there the determining factors. In: Waterhouse A.L., Kennedy J.A. (eds.) *Red wine color: revealing the misteries.* ACS Symposium series 886. 68-88.
- Garofano A., Lombardi D.S., Cedroni A., Tiberi D. (2003). Vinificazione per “differimento estrattivo” di uve rosse ad elevata presenza di antociani disostituiti. *Ind. Bevande.* 32. 4. 113-120.
- Gerbi V., Zeppa G., Rolle L., Ubigli M., Alessandria F. (2000). Aspetti enologici e sensoriali: In: *Barolo – Studio per la caratterizzazione del territorio, delle uve e dei vini dell'area di produzione.* Quaderni Regione Piemonte. 24: 75-86.
- Gerbi V., Zeppa G., Rolle L. (2002). Evoluzione delle antocianine nel corso della vinificazione delle uve 'Nebbiolo'. In: Porretta S. (eds), *Ricerche e innovazioni nell'industria alimentare, V*, Chiriotti Editori, Pinerolo TO, I, 420-427.
- Gerbi V., Cagnasso E., Caudana A. (2004) Tradizione e innovazione nella produzione divini a base 'Nebbiolo'. *Atti Convegno Nebbiolo Grapes.* Sondrio.
- Gerbi V., Rolle L., Zeppa G., Guidoni S., Schneider A. (2005). Indagine sul profilo antocianico di vitigni autoctoni piemontesi. *Industrie delle Bevande*, 34: 23-27.
- Gerbi V., Rolle L., Zeppa G. (2006). La vinificazione del Nebbiolo. *Quaderni di Scienze Viticole ed Enologiche.* 28: 167-187.
- Glories Y. (1984). La couleur des vins rouges. 2me partie. Mesure, origine et interprétation. *Conn. Vigne Vin.* 18. 253-271.
- Grazietti A. (2016). Impiego di chiarificanti e stabilizzanti in enologia. Seminario Università di Pisa. 9-104.
- Guidoni S., Allara P., Schubert A. (2002). Effect of cluster thinning on berry skin anthocyanin composition of *Vitis vinifera* cv Nebbiolo. *Am. J. Enol. Vitic.*, 53, 224-226.
- Habertson J. F., Picciotto E. A., Adams D. O. (2003). Measurement of polymeric pigments in grape berry extracts and wines using a protein precipitation assay combined with bisulfite bleaching. *American journal of enology and viticulture.* 54. 301-306.
- Hagerman A.E., Butler L.G. (1978). Protein precipitation method for the quantitative determination of tannins. *Journal Agricultural Food Chemistry.* 26. 809-812.

Ma W., Waffo-Teguo P., Jourdes M., Li H., Teissedre, P. L. (2016). Chemical affinity between tannin size and salivary protein binding abilities: Implications for wine astringency. *PloS one*. 11(8).

Mainardi G., Berta P. Il Piemonte della vite e del vino: un patrimonio di storia, tradizioni e territorio. Ricerca storica per la candidatura Unesco del Piemonte come patrimonio dell'umanità. OICCE. 1-8.

Mainardi G. (2004). Il podere reale di Pollenzo, centro di sperimentazione enologica nel Piemonte dell'Ottocento. L'attività di Paolo Francesco Staglieno, enologo di Carlo Alberto. Una città romana per una "real villeggiatura" romantica. *L'artistica Editrice*. 127-136.

Mainardi G. (2004). Il vino piemontese nell'Ottocento. Edizioni dell'Orso. Torino.

Mannini F., Argamante N., Ferrandino A., Guidoni S., Praz G., LaleDemoz P., Dozio S. (2006). Field performances and wine quality of three clones of Nebbiolo (*Vitisvinifera* L.) grown in two different environments of Northern Italy: Aosta Valley and Langhe. First international congress of mountain and steep slope viticulture. 1-7.

Moutounet M., Feuillat F., Puech J.L. (2003). Caractéristiques du bois de chêne en relation avec la qualité des vins. *Quaderni di Viticoltura ed Enologia*. Università di Torino. 26: 101-120.

Nuvolone G. (1798). Sulla coltivazione delle viti. *Calendario Georgico della Società Agraria di Torino*.

Ozguven A., Ozcelik Y. (2013). Investigation of some property changes of natural building stones exposed to fire and high heat. *Construction and building materials*. 38: 813-821

Ribereau-Gayon P., Dubourdiou D., Donèche B., Lonvaud A. (2005). *Handbook of Enology vol 1. The Microbiology of wine and vinifications*. Eds. John Wiley & Sons Ltd.

Ribereau-Gayon P., Glories Y., Maujean A., Dubourdiou D. (2005). *Handbook of Enology vol 2. The chemistry of wine stabilization and treatments*. Eds. John Wiley & Sons Ltd.

Robichaud J. L., Noble A. C. (1990). Astringency and bitterness of selected phenolics in wine. *Journal science food agriculture*. 53: 343–353.

Rolle L., Letaief H., Zeppa G., Gerbi V. (2006). Texture characteristics appraisal of mountainous Nebbiolo grapes. *Actes 1° Congrès International sur la viticulture de montagne et en forte pente, Saint-Vincent AO, I, 17-18 Marzo*, 79-80.

Rolle, Comunicazione personale, lezioni di Innovazione Tecnologica in Enologia. Anno accademico 2017/2018.

Schneider, A., Boccacci, P., Torello Marinoni, D., Botta, R., Akkak, A., Vouillamoz, J. (2004). Variabilità genetica e parentele inaspettate del Nebbiolo. *Proceedings of the International symposium Nebbiolo grapes*. 1-4.

Schneider A., Torello Marinoni D., Boccacci P., Botta R. (2006). Relazioni genetiche del vitigno Nebbiolo. *Quaderni di scienze viticole ed enologiche*. 28: 93-100.

Schneider A. (2017). Raccontare i vitigni dentro ai vini: alcune verità, molte bugie. "Comunicare il vino". *Convegno scientifico ONAV*. 1-20.

Somers, T.C. (1971). The polymeric nature of wine pigments. *Phytochemistry* 10. 2175-218.

Timberlake, C.F., and P. Bridle. (1976). Interactions between anthocyanins, phenolic compounds, and acetaldehyde and their significance in red wines. *American Journal of Enology and Viticulture*. 27: 97-105.

Ubigli M., Rissone M., Gerbi V., Anfosso P. (1991). Analisi strumentali e sensoriali del Barbaresco D.O.C.G.. *Rivista Viticola ed Enologica*. 2: 47-65.

Villamor, R.R., Harbertson, J.F. & Ross, C.F. (2009). Influence of tannin concentration, storage temperature and time on chemical and sensory properties of Cabernet Sauvignon and Merlot wines. *American Journal of Enology and Viticulture*. 60(4): 442-449.

Yayuan T., Weixi C., Baojun X. (2015). Profiles of phenolics, carotenoids and antioxidative capacities of thermal processed white, yellow, orange and purple sweet potatoes grown in Guilin, China. *Food Science and Human Wellness*. 4: 123-132.

Zeppa G., Rolle L., Gerbi V. (2008). Chemical and sensory characterisation of Nebbiolo VQPRD wines from North Piedmont. Segundo congreso internacional sobre viticultura de montana y en fuertependiente. CERVIM. 3-8.

SITOGRAHY

<http://www.abbaino.com/ivrea-e-non-solo/via-francigena/>

<https://www.aislombardia.it>

<http://www.cantinasperimentale.it/>

http://www.caremadoc.it/architettura_topiaria.php?lang=it

<http://www.cervim.org/viticultura-eroica.aspx>

<https://www.fulviovilla.com/tag/cmyk/>

<https://www.google.it/maps>

<http://www.oicce.it/sito/ot/ot26/ot26vigna.html>

<http://www.oiv.int/it/organizzazione-internazionale-della-vigna-e-del-vino>

<http://www.seminarioveronelli.com/sulle-presunte-origini-retiche-del-nebbiolo/>

<https://www.viefrancigene.org/it/>

<http://www.winereport.com/wineneWS/scheda.asp?IDCategoria=5&IDNews=1146>



UNIVERSITY OF TURIN

Department of Agricultural, Forest and Food Sciences

Largo Paolo Braccini, 2 - 10095 Grugliasco (TO) IT

STATEMENT

The student Vittoria Brugnago, made the changes suggested in her Master Dissertation entitled "Characterization of Nebbiolo wines in province of Turin and in lower Aosta valley", requested by the jury of the Master defence that took place in Lisbona on December 10th, 2019.

The final version of the Master Dissertation, I think, meets the required conditions to be delivered at the University of Lisbon.

Torino, January 15, 2020

The first supervisor

Prof. Vincenzo Gerbi

A handwritten signature in blue ink, appearing to read "Vincenzo Gerbi".