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The Influence of Traditional and Immobilized Yeast on the Amino-Acid Content of Sparkling Wine

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Abstract: This article focuses on the effect of yeast strains, vintage, and must sugar content on the amino-acid content of sparkling wines produced by the traditional method. In the experiment, the amino-acid concentrations before and after secondary fermentation, depending on the type of yeast used (basic wine without secondary fermentation, wine fermented with immobilized yeast, and wine fermented with classical Champagne yeast) and the sugar content of the must (170, 190, and 210 g per liter), and the vintage (2010 and 2011), were evaluated. Concentrations of 20 free amino acids in 18 wine variants were analyzed by ion-exchange liquid chromatography with postcolony ninhydrin derivatization and photometric detection. Results of the study show an increase in all the amino acids represented, except ornithine, after secondary fermentation. The average content of each amino acid in the basic wine, wine fermented with immobilized yeast, and wine fermented with classical Champagne yeast was higher in the variant where classical yeast was used. In this variant, the concentrations of alanine, glutamic acid, lysine, arginine, phenylalanine, valine, and glycine were almost twice as high as in the other variants. A higher proportion of most amino acids was observed in the year 2011; only for amino acids lysine, leucine, phenylalanine, tyrosine, ornithine, histidine, and methionine was a higher concentration observed in the year 2010. A higher concentration of released amino acids was also observed in wine produced from must with a higher sugar content (21° NM).

Keywords: traditional method; amino acid; sparkling wine; secondary fermentation

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1. Introduction

The yeast involved in secondary fermentation is selected on the basis of various analytical and technological criteria, including resistance to ethanol concentration, pressure, and temperature, high flocculation capacity, and good autolytic and foam properties [1,2]. During the maturing process, yeast autolysis [3] and various compounds can modify the sensory properties of the wine. Nitrogen compounds are generally considered to be the main compounds released into wine during autolysis [4]. The content of nitrogen compounds in sparkling wines, especially amino acids and peptides, is affected by the variety and time of lying on yeast lees [5,6]. Leroy et al. [7] found differences between wines processed by two strains of yeast traditionally used in the vinification of sparkling wine.

Lying on the yeast lees is used in many special wines. One example is the production of sparkling wines by the classic method of ripening in the bottle. In this case, the wine is

left in contact with its sludge for many months. Released amino acids are precursors of aromatic compounds such as higher alcohols, lactones, and polyamides [8].

Amino acids are precursors of a wide range of aromatics such as higher alcohols and, subsequently, also esters [9,10]. No differences in free amino-acid concentration have been observed between 3 and 9 months after secondary fermentation, irrespective of the grape variety selected. After 9 months, the free amino-acid concentration increased, which indicates the beginning of [11] autolysis.

Long maturation results in the release of sulfur compounds through decomposition of amino acids such as cysteine and methionine. Because of this, the older segments have an aroma associated with the content of released sulfur compounds, such as roasted nuts or coffee. Amino acids are subjected to secondary and tertiary reactions with fats and further development of taste and aroma. Differences in free amino-acid content, depending on yeast strain and ripening time, were observed, and significant differences in amino-acid content were detected. Exceptions were glutamine and methionine, which are not significantly affected by the maturation time on the lees [11–13]. The concentrations of aspartic acid, glutamine, histidine, threonine, arginine, ethanolamine, methionine, leucine, and tryptophan were not significantly affected by the yeast strain used. The differences between the basic wine and the wine after 20 days of secondary yeast fermentation showed that the amino acids—aspartic acid, asparagine + serine, histidine, gamma-aminobutyric acid, tyrosine, valine, isoleucine, tryptophan, and lysine—were reduced. Between 20 and 365 days of ripening in the bottle, there are significant differences in the representation of each amino acid. Asparagine, asparagine + serine, histidine, threonine, alanine, arginine, tyrosine, valine, isoleucine, leucine, lysine, and tryptophan increased their content, unlike glutamic acid, gamma-aminobutyric acid, and ethanolamine [2,7,8,12,14].

Some of these amino acids undergo transformation due to decarboxylation and deamination. Not only amino acids but also other nitrogen compounds are obtained during autolysis—polypeptides, peptides, and nucleic acids [8,15].

This study compares the effect of classical yeasts intended for secondary fermentation with immobilized yeasts in a 2 year experiment to a control sample of base wine. In addition to the influence of the vintage, the influence of the sugar content of the must from which the wine was produced was also evaluated. In a total of 18 wine variants, 20 individual amino acids were examined after 24 months of wine maturation.

The same yeast strain was used in both variants, where the patented MLC® technology for fermentation of sparkling wine without turbidity was used in the immobilized variant. Thanks to the sophisticated immobilization technology, it is possible to produce sparkling wine in accordance with traditional fermentation in the bottle without the need for shredding sludge. Yeast cells are deposited in alginate and, although they are highly active, they cannot enter the sparkling wine during fermentation or storage.

2. Materials and Methods

2.1. Biological Material

The experiment was carried out with grapes of the variety Pinot blanc (PB) harvested in 2010 and 2011 from a vineyard in Velké Němčice (Czech Republic), Punta (186 m). These are productive vineyards 7 to 9 years old, which are treated in the form of integrated grape production. The area is characterized as warm and dry with mild winters.

The grapes were harvested at so-called technological ripeness when they contained 170, 190, and 210 g of sugar (sugar content 17, 19, 21° NM) and 8 to 11 $g \cdot L^{-1}$ of acids.

From each variant, a micro-sample of wine with a volume of 15 L was produced by standard technology for the production of white still wines. The wines were treated with a dose of 50 $mg \cdot L^{-1}$ sulfur dioxide. Classical training and diatomaceous earth filtration (preparation for secondary fermentation) were performed on these samples. All microsamples were treated with tirage liqueur to a value of 24 $g \cdot L^{-1}$ of residual sugar. After secondary fermentation of the wine, it matured in a bottle for 36 months on yeast,

before disgorging without dosage liqueur. From each variant, a sample of the base wine was left for analytical and sensory evaluation.

2.2. Experiment Design

The prepared samples were then divided into three varieties: (1) left as a basic wine without secondary fermentation; (2) wine with the addition of immobilized yeast Cremanti (*Saccharomyces cerevisiae* Killer) (Institut nologique de Champagne, Mardeuil, France); (3) wine with added Champagne yeast "Champagne IOC 18-2007" (*Saccharomyces cerevisiae* Killer) ((La Littorale Enologia SL, Spain)).

The experimental diagram shows the breakdown by variety, vintage, sugar content of the must, and wine type (Figure 1).

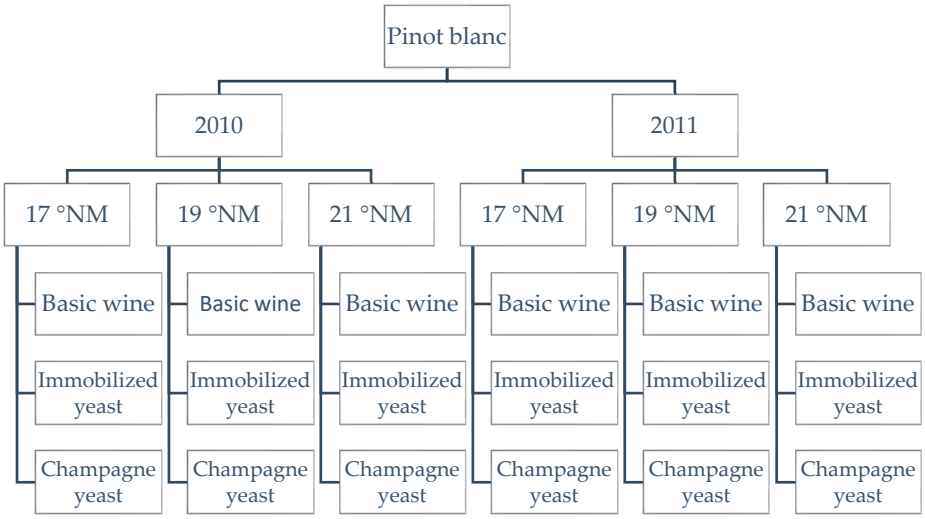

Figure 1. Experiment design.

2.3. Basic Characteristics of Immobilized Yeast 'Cremanti'

Cremanti® yeast are selected pure yeasts of *Saccharomyces cerevisiae* Killer for sparkling wine. They are immobilized according to the patented MLC® technology for fermentation of sparkling wine without turbidity. Cremanti® is dosed into each bottle before or after the addition of the cuvée. Thanks to the sophisticated immobilization technology, we can produce sparkling wine in accordance with traditional fermentation in the bottle without the need for shredding sludge. For immobilization, only legally authorized alginate is used. Yeast cells are deposited in alginate and, although they are highly active, they cannot enter the sparkling wine during fermentation or storage. The use of Cremanti® is authorized in accordance with applicable laws and standards. The cleanliness and quality are verified by a dedicated laboratory.

By using Cremanti®, it is possible to reduce costs during the entire process of producing sparkling wine by traditional bottle fermentation. This will improve the process of shaking, save space, and achieve high flexibility in terms of availability of freshly fermented and still unsweetened sparkling wine. The special immobilization process produces a sparkling wine free of fermentation (masks) during the entire fermentation process and during storage, provided that the appropriate wine base has been used. Complete yeast metabolism takes place inside immobilization beads.

2.4. The Classic Champagne Yeast IOC 18-2007

Champagne yeast is produced by 'Intitut oenologique de Chamapagne' under the designation IOC 18-2007-*Saccharomyces cerevisiae* Killer. This strain allows the production of very fine wines, fully preserving the characteristics of the variety and the peculiarities of the soil composition and climatic influence of the site (terroir). These yeasts are characterized by rapid and regular fermentation of sugars. Regular fermentation or fermentation at

low temperatures is recommended for sparkling wines. The recommended dosage for the production of sparkling wines in the bottle is 10–20 g·hL^{-1}.

They are characterized by high alcohol conversion (16 g of sugar produces 1% alcohol), low volatile acid production, high alcohol resistance (more than 15%), active Killer factor, good glycerol production (6 g·L^{-1}) (>20 million cells·g^{-1}), high purity (less than 10 wild yeast per million), and high purity (>10 million yeasts per million cells).

2.5. Characteristics of the Vintage

2.5.1. Vintage 2010

The vintage 2010 as a whole was, in terms of temperature, within the limits of the long-term average set for the period 1961 to 2000. In terms of precipitation, the vintage 2010 was one of those with above-average totals. In addition to the relatively high number of days with precipitation, especially in the spring months, their harmfulness was manifested by inappropriate timing due to the growth stages of the vine. For the whole year, it rained at individual locations from about 600 mm to more than 800 mm of precipitation. From the beginning of the year to the end of April, precipitation was mostly within normal limits. A significant amount of precipitation fell in May, and, until mid-June, there was a period with above-normal total precipitation, when 40 or more millimeters of rain occurred once. This was followed by a period of approximately 1 month with lower total precipitation. From mid-July, deviations from normal began to increase again; from August to the end of vegetation, they did not increase [16].

2.5.2. Vintage 2011

The wintering of vine bushes in 2010/2011 did not take place completely optimally. The vine began the phenomenon of sprouting around 10 April. The subsequent shorter but warm period hastened the vegetation. The vines began to bloom in early June, and flowering lasted for about 3 weeks. The subsequent course of vegetation was favorable for the vine, and it was a relatively dry year. Thanks to the excellent weather, the harvesting of grapes began in the first week of September. Due to the influence of the dry year, there was a rapid decrease in acids in the grapes, whereby many varieties had to be harvested a few days earlier. Most of the grapes were harvested by the end of October, depending on the processing capacity of the wineries [17].

2.6. Determination of the Content of Free Amino Acids and Some of Their Derivatives

Individual amino acids were determined using a previously published HPLC method [18]. Prior to analysis of the free amino-acid content, beverage samples were diluted with lithium nitrate buffer, and the aliquot was filtered through a 0.45 µm filter. Each sample was diluted in duplicate. The free amino-acid content was analyzed by ion-exchange liquid chromatography with postcolony ninhydrin derivatization and photometric detection (AAA 400, Ingos, Prague, Czech Republic). The following amino acids and their derivatives (hereinafter referred to as amino acids) were determined: threonine, serine, aspartic acid, glutamic acid, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, gamma-aminobutyric acid, ornithine, lysine, histidine, arginine, and amino-adipic acid. The results were expressed as milligrams of amino acids and their derivatives per liter of wine sample [18].

2.7. Statistical Evaluation

Commercially available software STATISTICA (StatSoft CR s.r.o., Zličín, Czech Republic) version 12.0 was used for data processing of the results. The comparison graphs show the average values of the individual variants. The interval of the standard error of the mean was chosen as a comparative sign of the significance of the influence of factors. For better clarity of some graphs (amino acids, phenolics, and volatile substances), a logarithmic scale was chosen for the *y*-axis. Verification of data normality (the verification did not confirm the normality of data, and non-parametric tests were chosen) had no assumptions about

the distribution of data. The Spearman coefficient was chosen for correlation comparison. The Spearman correlation coefficient follows the significance of the correlation between the two features (x, y). For both characteristics according to the size of the order (i) and from the pairs of order (i_x, i_y), we calculate the Spearman correlation coefficient (r_s) according to the following relation:

$$r_s = 1 - \frac{6 \sum_{i=1}^{n} (i_x - i_y)^2}{n \cdot (n^2 - 1)}$$

All correlations marked in red, whether positive or negative, are statistically significant at $\alpha = 0.05$. A positive correlation coefficient expresses a positive correlation between quantities, while a negative correlation coefficient expresses a negative correlation between quantities. If the value of the correlation coefficient is zero, there is no correlation between quantities (Table 1).

Table 1. Correlation values in absolute value and interpretation of the degree of dependence.

Correlation Value in Abs. Value	Interpretation of Dependence
0.01–0.09	Trivial, none
0.10–0.29	Low to medium
0.30–0.49	Medium to substantial (strong)
0.50–0.69	Substantial (strong) to very strong
0.70–0.89	Very strong
0.90–0.99	Almost perfect

3. Results

In the experiment, 18 samples were compared under large-scale winemaking conditions and using the same basic wine (Pinot blanc). The total free amino-acid content was determined in wines after 24 months maturation by high-performance liquid chromatography (HPLC).

3.1. Comparison of Amino-Acid Content before and after Secondary Fermentation

In studies by Torresi et al. [19,20] and Riberau et al. Ribéreau-Gayon, the following 23 amino acids were identified for Champagne sparkling wines: alanine, arginine, asparagine, aspartic acid, beta-alanine, citrulline, cysteine, gamma-aminobutyric acid, glutamic acid, glutamine, glycine, histidine, leucine, isoleucine, lysine, methionine, ornithine, phenylalanine, proline, serine, threonine, tyrosine, and valine [19,20]. Riberau et al. [20] stated the representation of individual amino acids in grapes and total amino acids, which are in the range of 1760–4590 mg·L^{-1}, depending on the ripeness of the grapes used for the production of sparkling wines in Champagne.

Figure 2 shows the average contents of each amino acid before and after secondary fermentation. From this comparison, an increase is apparent for all the amino acids represented, except ornithine (whose content did not change with secondary fermentation). Proline is not shown in the graph because its proportion did not change as the yeast does not assimilate it in the absence of oxygen.

The Bozdogan and Canbas study [14] focused on the analysis of 20 free amino acids. In this study, they found significant differences in amino-acid content during the maturation of the wine as a function of the slurry (time factor) and also of the yeast strain. Exceptions were glutamine and methionine, which are not significantly affected by aging. Aspartic acid, glutamine, histidine, threonine, arginine, ethanolamine, methionine, leucine, and tryptophan were not significantly affected by the yeast strain used. The differences between basal wines and wines after 20 days of secondary fermentation showed a decrease in aspartic acid, asparagine, serine, histidine, gamma-aminobutyric acid, tyrosine, valine, isoleucine, tryptophan, and lysine. Between 20 and 365 days maturation in the bottle, there were significant differences in aspartic acid, asparagine, serine, histidine, threonine, alanine,

arginine, tyrosine, valine, isoleucine, leucine, lysine, tryptophan, gamma-aminobutyric acid, and ethanolamine, whose content decreased [14].

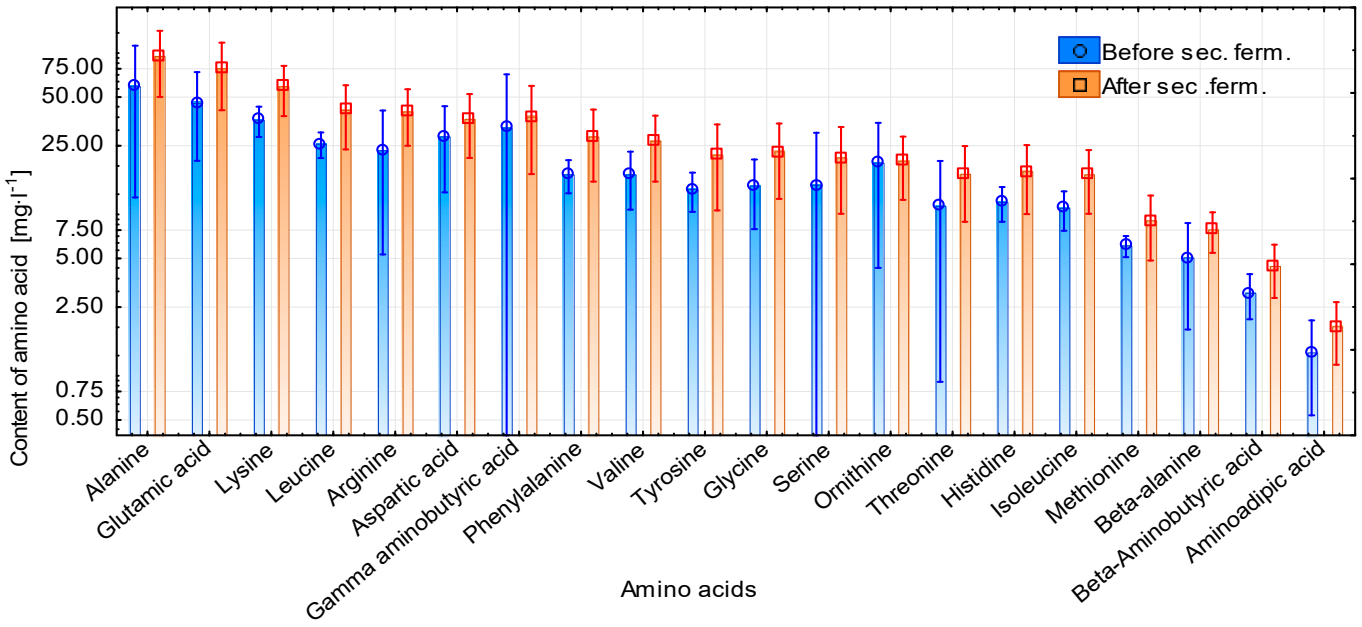

Figure 2. Comparison of average amino acid content categorized before and after secondary fermentation.

3.2. Comparison of Amino-Acid Content by Type of Wine

Scientific studies [14,21–24] of sparkling wines mainly focused on a comparison of the amino-acid content in connection with the maturation time after secondary fermentation. However, the studies have so far focused on minimizing the amount of amino acid, depending on the use of immobilized yeast and classical yeast with basic wine.

Figure 3 compares the average content of each amino acid in the basic wine, wine fermented with immobilized yeast, and wine fermented with classical Champagne yeast. In the graph, we can see the tendency for the majority of amino acids to increase in the variant where classical yeast was consumed. Alanine, glutamic acid, lysine, arginine, phenylalanine, valine, and glycine were the only amino acids in the wine with an almost twofold increase compared to the basic wine and the wine made using immobilized yeast. However, Figure 3 shows the fundamental differences between immobilized yeast and classical yeast in favor of a higher amino-acid representation in wines made using classic yeast. Bozdogan and Canbas [14] in their study stated that no significant differences were observed between the use of immobilized and classical free yeasts in the case of free amino acids and amino acids in peptides.

3.3. Comparison of the Amino-Acid Content by Must Sugar Content

Larger differences in this study were observed between yeast strains and between years. Comparison of the amino-acid content according to the sugar content of the must (Figure 4) shows a higher proportion of amino acids, excluding ornithine, in favor of higher sugar content (in this case, 21° NM). The wine produced from must sugar concentrations of 17° NM and 19° NM had approximately the same amino-acid content. The influence of the year on the representation of individual amino acids is shown in Figure 5, which shows a higher proportion of amino acids in the year 2011. Thus, it can be stated that the amino-acid content depends on many factors that have both negative and positive effects, as confirmed by Desportes et al. [25].

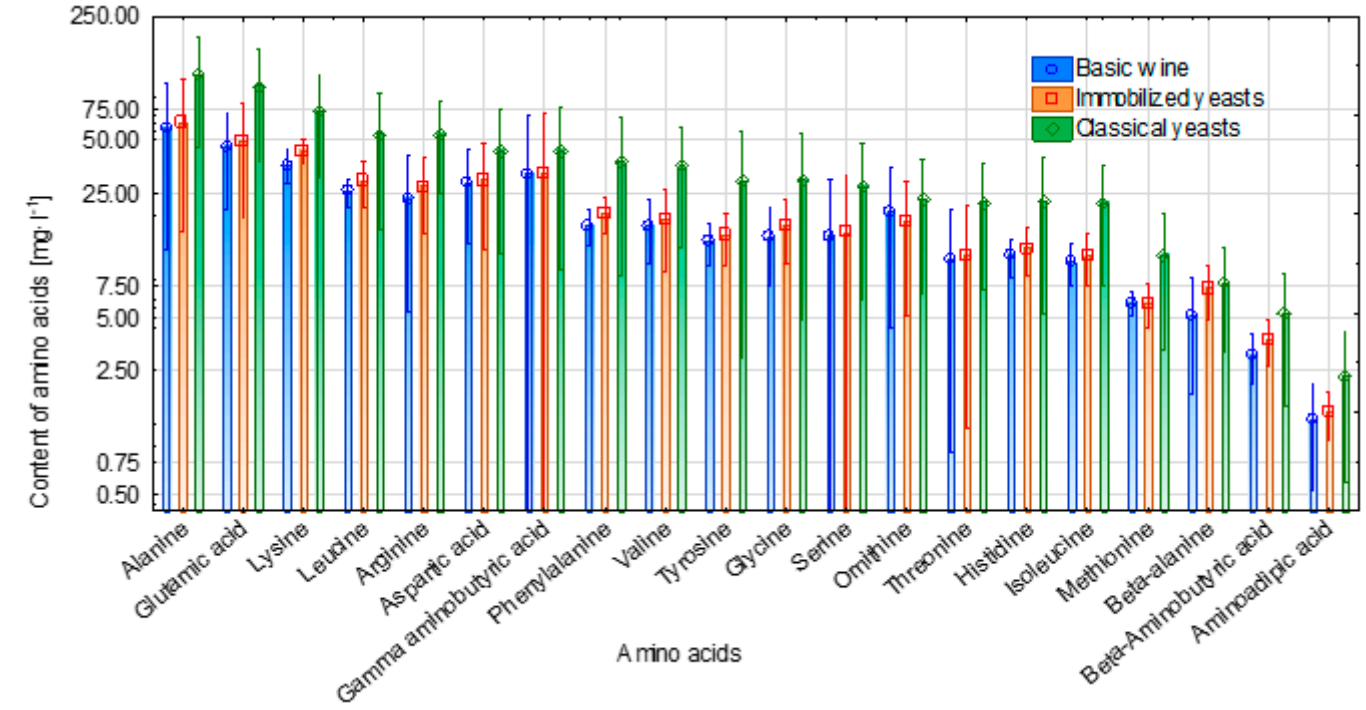

Figure 3. Comparison of the average amino-acid content of the basic wine, wine fermented with immobilized yeast, and wine fermented with classical yeasts.

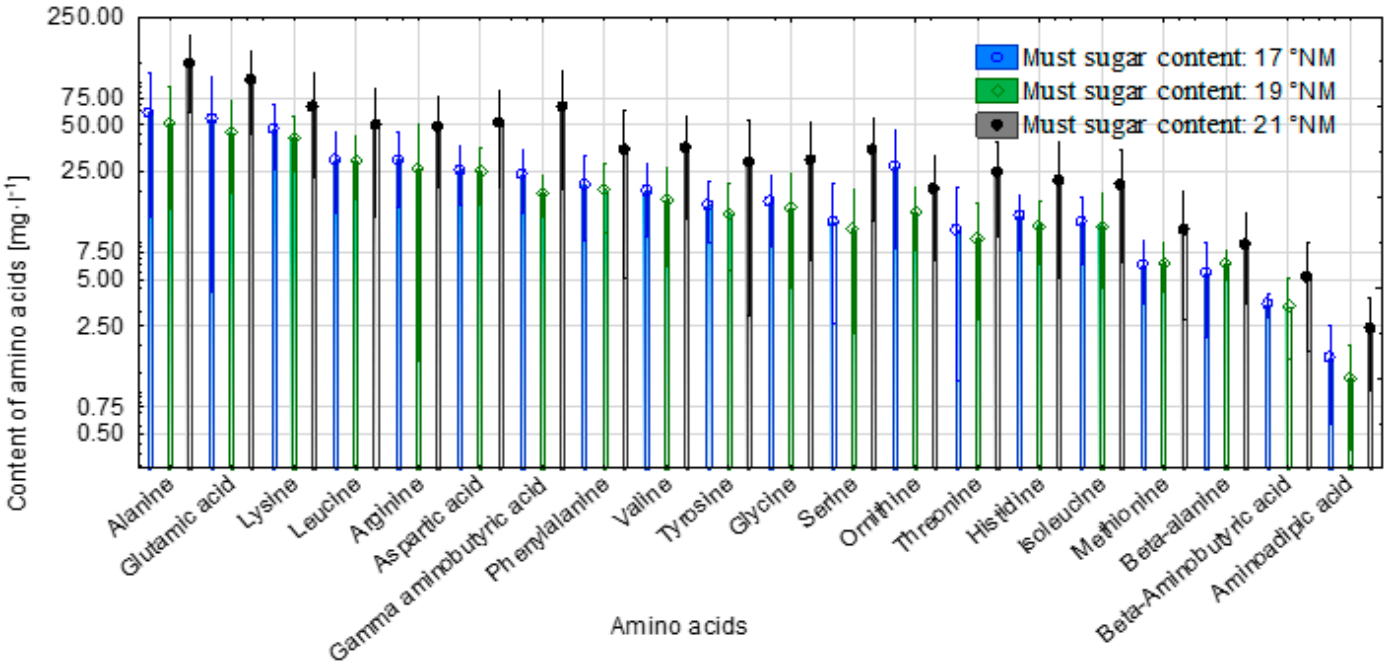

Figure 4. Comparison of average amino-acid content by must sugar content.

Figure 4 compares the average amino-acid content, categorized according to the sugar content of the must. In this division, the dominance of wines made from grape musts with a sugar content of 21° NM outside ornithine is noticeable. There was no appreciable trend in the wine grains with a sugar content of 17° NM having the lowest proportion, 19° NM having a higher proportion, and 21° NM having the highest proportion. Most amino acids had a content of 19° NM to 17° NM, with an even lower average content in some cases (alanine, glutamic acid, gamma-aminobutyric acid, lysine, and arginine).

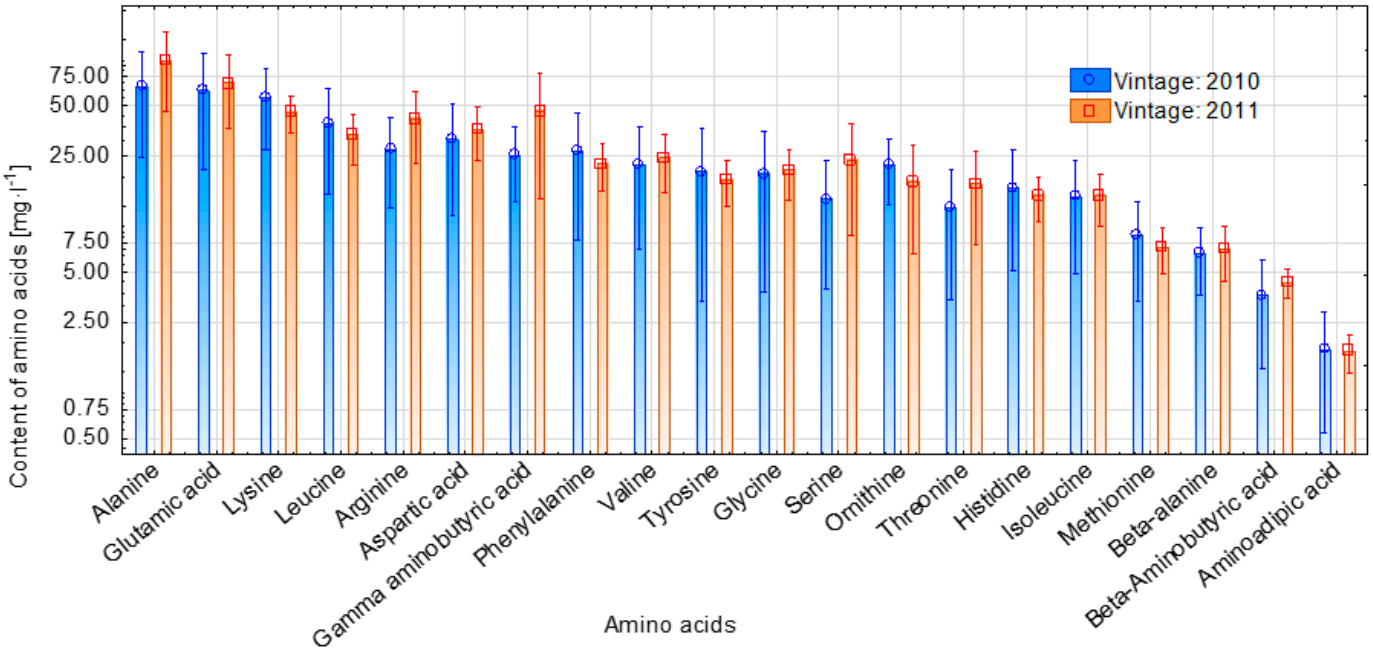

Figure 5. Comparison of average amino-acid content by year.

3.4. Comparison of Amino-Acid Content by Year

Figure 5 shows the average amino-acid content by year. In this case, the 2010 and 2011 years were compared. For 2011, the average content of amino acids apart from lysine, leucine, phenylalanine, tyrosine, ornithine, histidine, and methionine was higher. For these amino acids, the average content was higher in 2010.

3.5. Influence of Vintage, Sugar Content, Secondary Fermentation, and Yeast Type

For the purposes of this comparison, correlation matrices were produced showing statistically significant correlations between beta-aminobutyric acid and vintage and other correlations between the variants before and after secondary fermentation and lysine, as well as between wines made with immobilized and classical yeasts and lysine and arginine. These correlations ranged from substantial to very strong.

For the other amino acids represented, no statistically significant correlation with the vintage, must sugar content, variants before and after secondary fermentation, and between immobilized and classical yeasts was confirmed.

The influence of the year on the amino-acid content was demonstrated with a substantial to very strong dependence on beta-aminobutyric acid (Table 2). Secondary fermentation had a substantial to very strong effect on the lysine and classical yeast content and a substantial to very strong effect on lysine and arginine.

Table 2. The yeast correlation matrix comprising the secondary fermentation and yeast type with selected amino acids.

Variable	Lysine	Arginine	Beta-Aminobutyric Acid
Vintage	−0.032	0.332	0.524
Must sugar content	0.262	0.262	0.210
Before and after secondary fermentation	0.545	0.409	0.318
Immobilized or classic yeast	0.603	0.538	0.341

3.6. Amino Acids and Their Correlation Dependence

Another goal was to determine the dependence of each amino acid on the others. Correlation matrices showed a large proportion of positive statistically significant correlations between individual amino acids (Table 3). The values of the correlations between the individual amino acids ranged from 0.51 to 0.98, which can be interpreted as a substantial, very strong to almost perfect dependence (Table 1).

An exception was proline, a constituent of various types of wines, which does not change because it is not assimilated by yeast, as well as ornithine, which is produced by the hydrolysis of arginine. For these two amino acids, there was no positive or negative statistically significant correlation with other amino acids.

The protein content was found to be significantly correlated to parameters representative of foam stability, as were the amino acids arginine, asparagine, histidine, and tyrosine. Additionally, the production method was found to influence the foam collar height, which favored foaming in Méthode Traditionnelle wines over other those made by production methods. Understanding the contributions of key wine constituents to the visual and mouthfeel parameters of sparkling wine will enable more efficient production of high-quality wines [26]. In this research, from the point of view of dependencies of correlation matrices, the influence of the year on the amino-acid content was demonstrated with a substantial to very strong dependence on beta-aminobutyric acid. Secondary fermentation had a substantial to very strong effect on the lysine and classical yeast content and a substantial to very strong effect on lysine and arginine.

Table 3. Correlation matrices of the individual amino-acid dependencies among themselves.

Variable	Threonine	Serine	Aspartic Acid	Glutamic Acid	Proline	Glycine	Alanine	Valine	Methionine	Isoleucine	Leucine	Tyrosine
Threonine	1.00	0.95	0.84	0.93	0.09	0.87	0.90	0.94	0.78	0.93	0.84	0.89
Serine	0.95	1.00	0.82	0.89	0.18	0.87	0.89	0.91	0.74	0.93	0.85	0.87
Aspartic acid	0.84	0.82	1.00	0.81	0.18	0.92	0.66	0.82	0.83	0.82	0.84	0.94
Glutamic acid	0.93	0.89	0.81	1.00	−0.03	0.82	0.93	0.95	0.75	0.94	0.81	0.84
Proline	0.09	0.18	0.18	−0.03	1.00	0.04	0.01	−0.14	0.27	−0.09	0.17	0.03
Glycine	0.87	0.87	0.92	0.82	0.04	1.00	0.74	0.87	0.78	0.89	0.87	0.98
Alanine	0.90	0.89	0.66	0.93	0.01	0.74	1.00	0.88	0.61	0.87	0.70	0.73
Valine	0.94	0.91	0.82	0.95	−0.14	0.87	0.88	1.00	0.77	0.98	0.85	0.91
Methionine	0.78	0.74	0.83	0.75	0.27	0.78	0.61	0.77	1.00	0.78	0.92	0.83
Isoleucine	0.93	0.93	0.82	0.94	−0.09	0.89	0.87	0.98	0.78	1.00	0.89	0.91
Leucine	0.84	0.85	0.84	0.81	0.17	0.87	0.70	0.85	0.92	0.89	1.00	0.91
Tyrosine	0.89	0.87	0.94	0.84	0.03	0.98	0.73	0.91	0.83	0.91	0.91	1.00
Phenylalanine	0.84	0.83	0.89	0.80	0.12	0.93	0.67	0.85	0.89	0.87	0.98	0.96
Beta-alanine	0.55	0.54	0.70	0.35	0.39	0.69	0.32	0.41	0.59	0.43	0.60	0.66
Beta-aminobutyric acid	0.88	0.85	0.82	0.76	−0.09	0.93	0.75	0.86	0.67	0.86	0.77	0.89
Gamma aminobutyric acid	0.64	0.67	0.70	0.55	0.33	0.67	0.57	0.56	0.68	0.57	0.64	0.67
Ornithine	−0.24	−0.26	0.03	−0.09	−0.24	−0.07	−0.19	−0.14	−0.28	−0.20	−0.27	−0.04
Lysine	0.71	0.69	0.42	0.76	−0.21	0.59	0.83	0.75	0.56	0.74	0.67	0.60
Histidine	0.89	0.91	0.74	0.86	−0.01	0.85	0.83	0.91	0.81	0.92	0.90	0.87
Arginine	0.86	0.85	0.59	0.83	−0.12	0.74	0.92	0.85	0.60	0.85	0.70	0.73
Aminoadipic acid	0.86	0.83	0.93	0.88	0.02	0.94	0.75	0.90	0.78	0.88	0.85	0.95
Variable	**Phenylalanine**	**Beta-Alanine**	**Beta-Aminobutyric Acid**		**Gamma Aminobutyric Acid**		**Ornithine**	**Lysine**	**Histidine**	**Arginine**	**Aminoadipic Acid**	
Threonine	0.84	0.55	0.88		0.64		−0.24	0.71	0.89	0.86	0.86	
Serine	0.83	0.54	0.85		0.67		−0.26	0.69	0.91	0.85	0.83	
Aspartic acid	0.89	0.70	0.82		0.70		0.03	0.42	0.74	0.59	0.93	
Glutamic acid	0.80	0.35	0.76		0.55		−0.09	0.76	0.86	0.83	0.88	
Proline	0.12	0.39	−0.09		0.33		−0.24	−0.21	−0.01	−0.12	0.02	
Glycine	0.93	0.69	0.93		0.67		−0.07	0.59	0.85	0.74	0.94	
Alanine	0.67	0.32	0.75		0.57		−0.19	0.83	0.83	0.92	0.75	
Valine	0.85	0.41	0.86		0.56		−0.14	0.75	0.91	0.85	0.90	

Table 3. *Cont.*

Variable	Phenylalanine	Beta-Alanine	Beta-Aminobutyric Acid	Gamma Aminobutyric Acid	Ornithine	Lysine	Histidine	Arginine	Aminoadipic Acid
Methionine	0.89	0.59	0.67	0.68	−0.28	0.56	0.81	0.60	0.78
Isoleucine	0.87	0.43	0.86	0.57	−0.20	0.74	0.92	0.85	0.88
Leucine	0.98	0.60	0.77	0.64	−0.27	0.67	0.90	0.70	0.85
Tyrosine	0.96	0.66	0.89	0.67	−0.04	0.60	0.87	0.73	0.95
Phenylalanine	1.00	0.68	0.83	0.67	−0.17	0.63	0.89	0.68	0.90
Beta-alanine	0.68	1.00	0.69	0.60	−0.15	0.24	0.53	0.36	0.58
Beta-Aminobutyric acid	0.83	0.69	1.00	0.60	−0.24	0.61	0.83	0.80	0.83
Gamma aminobutyric acid	0.67	0.60	0.60	1.00	−0.07	0.44	0.68	0.57	0.57
Ornithine	−0.17	−0.15	−0.24	−0.07	1.00	−0.30	−0.31	−0.31	0.03
Lysine	0.63	0.24	0.61	0.44	−0.30	1.00	0.83	0.88	0.57
Histidine	0.89	0.53	0.83	0.68	−0.31	0.83	1.00	0.86	0.79
Arginine	0.68	0.36	0.80	0.57	−0.31	0.88	0.86	1.00	0.67
Aminoadipic acid	0.90	0.58	0.83	0.57	0.03	0.57	0.79	0.67	1.00

4. Conclusions

This experiment offers an expanded view of the amino-acid content of sparkling wines in two years, produced in three different ways with three different sugar contents. The mean percentages of all analyzed amino acids increased after secondary fermentation. Significant differences in the amino-acid content of wines were found using immobilized yeast and classical yeast in favor of a higher percentage of amino acids in wines made using classical yeasts.

Larger differences in this study were observed between yeast strains and between years. The influence of the year on the representation of individual amino acids generally showed a higher proportion of amino acids in the year 2011; only for amino acids lysine, leucine, phenylalanine, tyrosine, ornithine, histidine, and methionine was the average content higher in 2010. Comparison of the amino-acid content according to the sugar content of the must showed a higher proportion of amino acids, excluding ornithine, in favor of higher sugar content (in this case, 21° NM). The wine produced from musts with sugar content of 17° NM and 19° NM had approximately the same amino-acid concentration. From these results, we can conclude that the amino-acid concentration depends on many factors that have both negative and positive effects.

Correlation matrices showed statistically significant correlations between beta-aminobutyric acid and vintage. Other correlations between the variants before and after secondary fermentation and lysine, as well as between wines made with immobilized and classical yeasts and lysine and arginine, ranged from substantial to very strong. For the other amino acids represented, no statistically significant correlation with the vintage, must sugar content, variants before and after secondary fermentation, and between immobilized and classical yeasts was confirmed. The influence of the year on the amino-acid content was demonstrated with a substantial to very strong dependence on beta-aminobutyric acid. Secondary fermentation had a substantial to very strong effect on the lysine and classical yeast content and a substantial to very strong effect on lysine and arginine.

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