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The stability of major and trace element concentrations from musts to Champagne during the production process

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ABSTRACT

Thirty-nine Champagnes from six different brands originating from the AOC Champagne area were analyzed for major and trace element concentrations in the context of their production processes and in relation to their geographical origins. Inorganic analyses were performed on the must (i.e., grape juice) originating from different AOC areas and the final Champagne. The observed elemental concentrations displayed a very narrow range of variability. Typical concentrations observed in Champagne are expressed in mg/L for elements such as K, Ca, Mg, Na, B, Fe, A, and Mn. They are expressed in µg/L for trace elements such as Sr, Rb, Ba, Cu, Ni, Pb Cr and Li in decreasing order of concentrations. This overall homogeneity was observed for Sr and Rb in particular, which showed a very narrow range of concentrations ($150 < Rb < 300$ µg/L and $150 < Sr < 350$ µg/L) in Champagne. The musts contained similar levels of concentration but showed slightly higher variability since they are directly influenced by the bedrock, which is quite homogenous in the AOC area being studied. Besides the homogeneity of the bedrock, the overall stability of the concentrations recorded in the samples can also be directly linked to the successive blending steps, both at the must level and prior to the final bottling. A detailed analysis of the main additives, sugar, yeast and bentonite, during the Champagne production process, did not show a major impact on the elemental signature of Champagne.

KEYWORDS: Champagne, winemaking process, elemental concentrations, soil, blending, stability, musts, oenological processes

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INTRODUCTION

The geographical origin of food and beverages has become increasingly important for consumers, as well as for producers and distributors. Food or beverage from a specific and protected area is more highly regarded when its geographical origin has been documented, thereby increasing its value and its chances of being sold at a higher price. Tracing geographical origin has become a priority, especially for renown products such as wines originating from a specific region or "terroir" (van Leeuwen and Seguin, 2006). In order to detect any adulteration of wines and possible origin fraud, the authorities in charge of the protected designation of origin are in need of precise and reliable scientific methods for tracing the geographical origin of grapes and the wine produced from them. Linking wines to their terroir of origin is a way of certifying genuineness. Scientists therefore need to meet the challenge of establishing unambiguous parameters for identifying the terroir and clearly define the geographical area of the grape.

The huge diversity of winegrowing areas is a major challenge when determining the provenance of wine. Indeed, the qualities and properties of wine are influenced by several factors, such as grape variety, soil, and climate, as well as by oenological practices, such as the yeast inoculum or other inputs used (van Leeuwen *et al*., 2006). In this context, the geogenic concentration of minerals or inorganic components of wines - particularly sparkling wines - can be considered an important variable for tracing the environment of the vine. There are two main sources of overall signature of inorganic elements in wine. One is directly related to the uptake of elements from the soil by the plant, which can accumulate in the grapes and be transferred to the wine, if they are not eliminated by the winemaking or clarifying processes. The other originates from external inputs, either from external contamination (such as atmospheric contamination, as in the case of lead (Pb) (Barbaste *et al*., 2001; Epova *et al*., 2020; Medina *et al*., 2000) or direct inputs to the winemaking process, which typically originate from the pulverization of the vines by pesticides (e.g., bio-pesticides, such as copper salt or "Bouillie Bordelaise"), or from the addition of specific products that are directly added to improve and control the winemaking process. However, they can also be released from the containers and packaging material. Such inorganic and mineral components of wines have already been used as a possible fingerprint for the characterization of the geographical origin of wines (Taylor *et al*., 2003). In general, the inorganic and mineral content can seem, at first, an effective signature of the geographical origin since it is closely linked to the composition of the soil on which vines are grown. However, this inorganic signature is influenced by several external factors arising after uptake from the soil: the addition of elements during the production of grapes in the vineyard, the winemaking process and/or storage (Kment *et al*., 2005; Pohl, 2007). Vineyard management practices can result in an increase in the concentration of elements, such as arsenic (As), cadmium (Cd), copper (Cu), manganese (Mn), lead (Pb) and zinc (Zn) (Fiket *et al*., 2011; Kment *et al*., 2005; Pohl, 2007). Lastly, a third source can also result from direct environmental pollution (Pohl, 2007), as well as different winemaking steps leading to the release of additional inorganic elements (intended or not) into the wine (e.g., aluminum (Al), Cd, chromium (Cr), Cu, iron (Fe) and Zn), resulting from, for instance, the long contact of wine with materials used in machine parts, pipes and casks (Cerutti *et al*., 2019; Fiket *et al*., 2011; Kment *et al*., 2005; Pohl, 2007).

All the aforementioned studies were performed on still wine. For sparkling wines, the production process is substantially different. Only the juice of whole bunches is recovered by the gentle pressing of the grapes. This procedure limits the contact between the skin and the juice, thereby minimizing the release of elements from skin to juice. The first alcoholic fermentation is followed most of the time by malolactic fermentation and another alcoholic fermentation in a glass bottle. In general, the sparkling wine is kept in the glass bottle in contact with the yeast for between 1 and 7 years. Hence, sparkling wine production involves more steps and time than still wine production.

Therefore, an a priori systematic study of the performance of the investigated indicators in all the steps of sparkling wine production (encompassing soil, plant, fruit, raw materials, must inputs, blending and finished product) comprises a holistic approach to developing a model for determining the geographical origin of the wine. The objective of this study was to improve knowledge on geographical traceability indicators taking into account the whole Champagne making process from the fruit to the sparkling wine. Multi-elemental signatures were monitored throughout the different steps of the Champagne sparkling winemaking process by analyzing the grapes, musts and other constituents for their elemental concentrations. Such analyses are a fundamental prerequisite for establishing a possible link between the final product and its geographical origin for future authentication of Champagne and identification of counterfeited products.

MATERIALS AND METHODS

1. Reagents

All sample preparation procedures were performed in an ISO 7 cleanroom. Ultrapure HNO_3 (67-70 %, ULTREX® II, J.T.Baker), Ultrapure H_2O_2 (67-70%, ULTREX® II, J.T.Baker) and Ultrapure water (18 $M\Omega cm^{-1}$) were used for digestion, purification and dilutions prior to measurements. The pre-concentration of samples, when necessary, was performed by evaporation in 15 or 30 mL PFA Savillex vials (Savillex Corporation, USA) under a fume hood. Sample evaporation was performed with closed evaporation device (Evapoclean, Analab, France). All vessels used were 15 mL and 30 mL Teflon vessels (Savillex, USA) and were washed with 10 % Ultrapure HNO_3 and 10 % Ultrapure HCl. The vessels were rinsed with Ultrapure water (18 MΩcm).

2. Standards and quality control

Quality control procedures were organized for each session of multielemental analysis with Quadrupole–Induced Coupled Plasma Mass Spectrometry (Q-ICPMS). The quality control procedures were performed by analyzing Certified Reference Materials using both a standard of river water SLRS5 (NRC Canada) and natural water NIST1640a (NIST USA). In-house reference material comprising Champagne (ChRM) was also analyzed in triplicate during each session to ensure the quality of the sample mineralization and the analysis. This Champagne reference material was produced in a previous study (Cellier *et al*., 2021). A series of calibration solutions were prepared in a range of 0.005 to 50 g/kg. During the ICP-MS analysis, a drift correction for ICP-MS was added and controlled using normalization to mid-mass elements, such as Yttrium (^{90}Y) and Rhodium (^{103}Rh).

3. Origin of the samples and sampling during Champagne production

In order to achieve a comprehensive assessment of total inorganic composition during all the Champagne production processes, samples were collected at each stage, from the musts (29 samples) and base wines (11 samples) to the final Champagnes bottles (different Champagne brands and different vintages whenever available; a total of 39 different bottles). The different crucial steps of Champagne production are shown in Figure 1. First, the grapes are gently pressed to expel the juice and to obtain the musts (grape juice). Then these musts are blended and left in the tank for alcoholic and malolactic fermentation (for approximately 3 weeks) to obtain the base wines, which are then blended to obtain the desired "*cuvées*". After these different steps, the base wines are bottled with the addition of yeast, bentonite, and sugars; these additions are necessary to promote and control the second alcoholic fermentation. At the end of fermentation, an "expedition liqueur", made of matured wine and sugar, is added in various proportions to achieve the desired Champagne sugar content before shipping.

In our study, to obtain a representative sample set in all the different steps of the winemaking process starting from the grapes, a collaboration with six local Champagne houses was established. With their support, a wide array of samples was collected at all the critical stages of the wine making procedure. It should be noted that the following analyses performed on musts cannot be directly related to a specific brand, because, as part of the traditional practices of each prestigious Champagne brand, must and base wines are often mixed during the Champagne making process, and this information is confidential.

The samples originated from large Champagne production companies. In general, Champagne produced by large brands involves the blending of wines from several different years. Different types of samples from different years and at different production stages were collected. These are detailed below and in Table 1 of Supplementary Materials:

- Must samples: during the 2018 harvest, 29 must samples were taken from all the AOC Champagne areas. Must is the juice of grapes after gentle pressing, from which, the skins, pips and stalks are removed immediately after pressing. Each of the 29 musts was collected from a specific and delimited wine growing area in the AOC Champagne region, and originated from one specific grape variety only (Chardonnay, Pinot noir or Meunier).

- Champagne samples: 39 Champagnes from 6 different brands of different vintages between 1983 and 2016, white and rosé, with indication of vintage or not, and brut and dry were collected and analyzed. All 39 Champagnes had been made from grapes produced by vines grown throughout

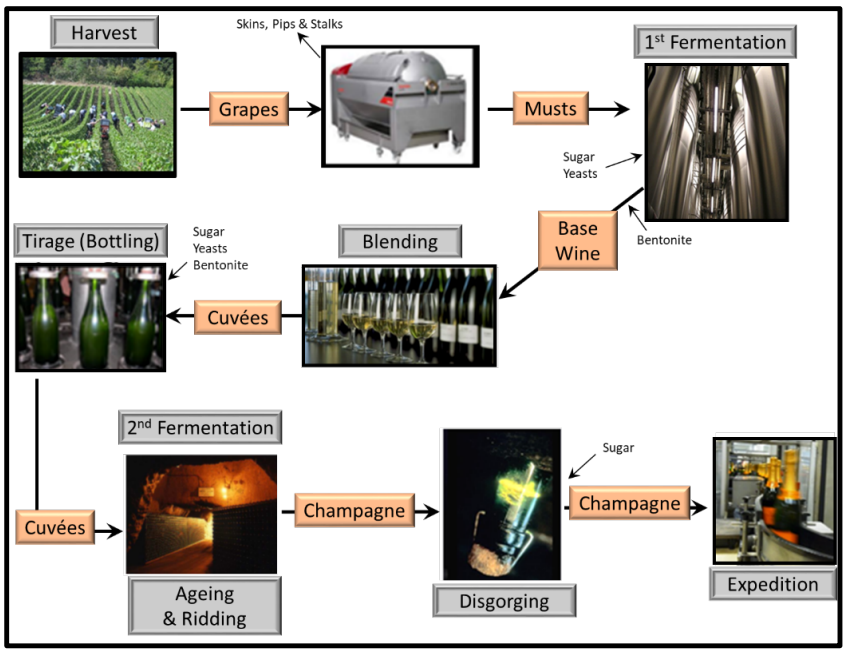


FIGURE 1. Simplified diagram of the production process of Champagne.

the AOC Champagne region. The Champagne is the final product obtained after the blending of base wines and bottling. During this process, two alcoholic fermentations take place and the Champagne is then left for ageing on the lees for, on average, between 2 and 3 years for non-vintage wines, and between 4 and 10 years for vintage wines.

Inputs collected during the process: in order to thoroughly monitor the inorganic signatures during the production process of Champagne, as well as the initial inputs (the juice of the grapes), all the potential additional inputs of the different steps of the process were analyzed. This was done by collecting and analyzing the raw material of the inputs used during Champagne production, such as samples of sugar, yeast and two types of bentonite used in Champagne. In general, sugar is added during the first and second alcoholic fermentations and at the end of the champagne making process in order to define champagne (with sugar content increasing from brut nature, extra brut, brut, extra dry, medium dry to sweet). Yeast is also added during the first and second fermentations. Bentonite is a specific clay which is added to the base wine to facilitate the clarification of the Champagne by inducing the settling and sedimentation of the particles in suspension. The bentonite used was a mixture of Ca and Na-bentonite.

4. Sample preparation

For the elemental analyses, all samples were first mineralized. For the mineralization steps of the liquid samples, such as the musts and Champagnes, 1 mL of sample was mixed with 1 mL of HNO_3 (67-70 %) and then heated at 80 °C with a Hot block (heating plate) for 4 h. Then 0.5 mL of H_2O_2 (a strong oxidation agent used to destroy the organic matter content) was added and the solution was heated at 80 °C for 2 h (Cellier *et al*., 2021). A High-Pressure mineralizer (HPA Anton Parr) operating at 300 °C and 300 Bars was used when higher energy mineralization conditions were required for solid samples that needed to be fully mineralized. By following these different digestion procedures, it was possible to always obtain very clear and fully dissolved samples from grape, sugar, yeast and bentonite. A total amount of 1 g maximum for full digestion (for security) of the solid samples was used in the High-Pressure vials, to which 5 mL of HNO_3 was added (at ambient temperature and pressure). After 5 h, 0.5 mL of H_2O_2 was also added, the digestion vials were closed again and the HPA cycle was turned on (4 h, 320 °C, 130 bar). Lastly, the samples were diluted 100-fold with ultra-pure H_2O and then analyzed with a Q-ICPMS.

5. Instrumentation and operating conditions

All the multielemental analyses were performed with an ELAN DRCII ICPMS. A 200 µL/min PFA ST nebuliser was connected to a 50 mL twister spray chamber with a Helix CT and was used for sample introduction. To remove any potential polyatomic interferences, a 0.7 mL/min of NH_3 gas flow was added for the analysis of Mg, Al, Mn, Ni, Cu, Cr, Fe, Ca and K as reaction gases. The other analytes (Li, B, Rb, Sr, Ba and Pb) were analysed in standard mode. The optimisation of the ICPMS was performed with a multielemental solution to obtain the highest sensitivity and stability and a low oxide level (below 3 %). The instrumental and data acquisition parameters are given in Table 1. The calibration standards solutions were prepared by successive dilution from mono-elemental solutions of Sr, Rb, Pb, Tl, K, Ca, Na and Mg (SCP science, Plasma CAL, Canada). These multielemental calibration standard solutions were analysed at the beginning and at the end of the session to correct for potential drift. The CRMs used were the NIST1640a Reference material from natural water, as well as an "in house Champagne reference material" made for the purpose (Cellier *et al*., 2021). This sample was regularly analysed

TABLE 1. Operating conditions of the ICPMS.

Elan DRCII (Perkin Elmer)	
Spray Chamber	Glass cyclonic
Interface	Nickel
RF Power	1,050 W
Operating Mode	Classic / Collision
Collision Gas	NH_3
Plasma Gas Flow	16 $L.min^{-1}$
Auxiliary Gas Flow	1 $L.min^{-1}$
Nebulizer Gas Flow	0.85 $L.min^{-1}$
Collision Gas Flow	0.7 $mL.min^{-1}$
Dwell Time	100 ms
Sample Uptake Rate	0.9 $mL.min^{-1}$
Number of replicates	3
Replicate time	12 s
Isotopes	On Classic mode: ^{85}Rb, ^{88}Sr, ^{208}Pb, ^{24}Mg and ^{23}Na On DRCII mode: ^{42}Ca and ^{39}K

TABLE 2. The elements monitored and the appropriate detection mode and associated limits of detection (LODs).

Element	m/z	Mode[a]	LOD[b] (µg/L)
Li	7	ng	0.355
B	11	ng	0.22
Rb	85	ng	0.003
Sr	88	ng	0.024
Ba	137	ng	0.015
Pb	208	ng	0.004
Mg	24	ng	1.37
Na	23	ng	0.595
Al	27	ng	0.7
V	51	NH_3	0.015
Mn	55	NH_3	0.012
Ni	60	NH_3	0.009
Cu	63	NH_3	0.021
Zn	66	NH_3	0.18
Cr	52	NH_3	0.009
Ca	43	NH_3	1.23
K	39	NH_3	0.67
Fe	56	NH_3	0.32

(after every 40 samples) to validate our results. A 6-point calibration method was performed and the LODs were calculated for all the elements and are listed in Table 2.

RESULTS AND DISCUSSION

1. Concentrations of inorganic elements in the Champagnes

Before implementing an in-depth study of the inorganic changes during the whole cycle of the Champagne-making process, the inorganic and trace element contents of the final 39 Champagne samples were determined.

The elemental analysis of the Champagne samples was performed by Q-ICPMS. Major elements of the inorganic content which were monitored were selected for their potential link with the soil composition (B, Na, Al, K, Mn, Fe and Sr). The analyzed trace elements were Cr, Ni, Cu, Rb, Ba, and Pb as shown in Table 3.

Two categories of elements can be identified with respect to their concentration ranges: those above 800 µg/L (such as K, Ca, Mg, Na, B, Fe, Al and Mn) and those below 800 µg/L (Sr, Rb, Ba, Cu, Ni, Pb, Cr and Li).

These inorganic analyses were performed on 39 different Champagnes (from 6 different brands and produced between produced 1983 and 2016). The results given in Table 3 clearly show that all the Champagne brands across a wide range of vintages contain very similar concentrations of inorganic elements. This general homogeneity is consistent with the overall good homogeneity of the samples. Indeed, the RSDs are all below 40 % for all elements (except for Pb as described in detail later), being remarkably low for B, Mg, and Rb (7 %, 17 % and 18 % respectively). These results are independent of harvest year and vineyard location. Hence, the analyzed Champagnes all have a similar multielemental signature; this homogeneous trend could be linked to the blending of musts operated during the production process of the Champagne.

When comparing the results with data from the literature, they were found to be quite different from the general overview proposed by Pohl (2007). In this study, the ranges of elemental concentrations of wines from different countries were reported, clearly showing that they can be very wide, even inside the same country (e.g., $0.06 < Fe < 11.49$ mg/L for wine in Australia compared to the narrow range of concentrations in the analyzed Champagne: $1.0 < Fe < 2.0$ mg/L). The concentrations of Sr and B in these Champagnes were also very homogeneous, but remarkably different from South African samples (Coetzee *et al*., 2014), which were found to have an average

TABLE 3. Average and median elemental (major and trace) concentrations in the 39 Champagnes.

N = 39	mg.L^{-1}							
	K	Ca	Mg	Na	B	Fe	Al	Mn
Average	415	82	75	12	2.84	1.6	0.9	0.8
Median	388	84	73	11	2.86	1.4	0.9	0.7
SD	104	19	13	4	0.2	0.4	0.2	0.2
RSD %	25 %	24 %	17 %	34 %	7 %	28 %	27 %	25 %
N = 39	µg.L^{-1}							
	Sr	Rb	Ba	Cu	Ni	Pb	Cr	Li
Average	260	216	50	36	24	17	16	3.9
Median	264	207	46	34	23	9	15	3.7
SD	54	39	14	8	9	18	4	1.3
RSD %	21 %	18 %	28 %	22 %	38 %	106 %	28 %	33 %

Sr concentration of 60 µg/L (i.e., 3.5 times less than that of Champagne) and an average B concentration of 650 µg/L (i.e., 4.5 times less than that of Champagne), which is mainly due to geological differences in these regions.

Special attention was paid to Sr and Rb content for two main reasons. First, Sr concentrations can generally be attributed to the "terroir" (bedrock and associated soil) and these concentrations are generally higher in wines produced on calcareous soils (Gupta *et al.*, 2018). In contrast, Rb concentrations are associated with more siliceous soils (Gupta *et al.*, 2018). The occurrence of Rb during the analytical process is always noteworthy since it can later affect the results of the analyses: it has strong potential for interfering with other elements as it has a similar isotopic mass. It can potentially influence the precision of the Sr isotopic ratio.

In Table 3 and in Figure 2 it is possible to observe the remarkable homogeneity of the elemental concentrations of Rb and Sr, regardless of the vintage (over 34 years) and geographical location (the sub-area within the whole AOC zone). The overall variability of the concentrations is relatively small and ranges between 170 and 350 µg/L for Sr and between 180 and 320 µg/L for Rb in all 6 brands of the 39 different Champagnes. Elements such as Rb and Sr are a good signature of soil composition, their concentration and availability depending on the geological age of the bedrock and soil type.

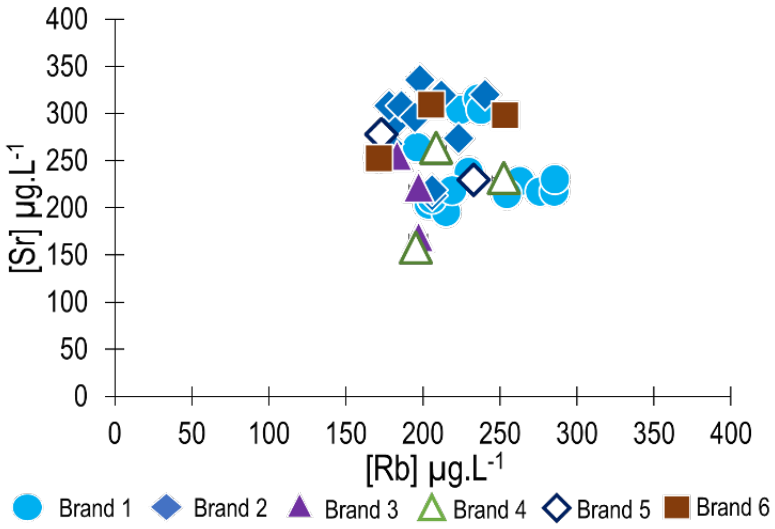


FIGURE 2. Sr and Rb concentrations in 39 Champagnes from 6 different brands. Error bars are smaller than the size of the dots.

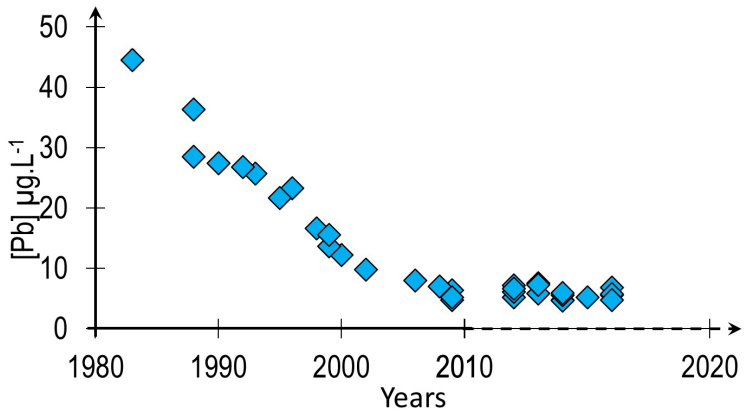


FIGURE 3. Inorganic concentrations of Pb in Champagne from 1983 to 2016. Errors bars are smaller than the size of the dots.

Pb concentrations (Table 3) were found to have a much higher RSD and dispersion compared to the other elements. The most noticeable observation is a steady decrease over the 40-year span of samples, starting from 40 µg/L in the 1980s down to approximately 5 µg/L in 2010, after which it stabilized. It is worth mentioning that a very wide array of vintages was analyzed (from 1983 to 2016) and the large RSD in Pb content of the Champagne reflects the considerable decrease in lead content of Champagne over the years. This has also been previously observed in other wine growing areas (Barbaste *et al*., 2001; Epova *et al*., 2020; Rosman *et al*., 1998). The old Champagne vintages contain higher concentrations of Pb, because during their time atmospheric contamination from leaded gasoline was still very present. Nonetheless, these Pb concentrations in Champagne are always much lower than the 100 µ/L level recommended by OIV since 2019.

2. Concentrations of inorganic elements in musts

The low variability of the total inorganic content of the Champagne produced throughout this area is not surprising, since the soil characteristics and the geology of the whole AOC Champagne area are quite homogeneous. The AOC Champagne area is located in the sedimentary basin around Paris. The Parisian basin was gradually formed layer by layer by sedimentation during several periods of transgression interrupted by episodes of sea retreat.

According to the geological map of the Marne and Aube department (Vernhet, 2007), chalky (calcareous) soils cover most of the Champagne wine region. More importantly, there are predominantly two soil types in the Champagne region: chalky soils from the Cretaceous period and marl soils from the Cenozoic period (Oligocene/Eocene). As a result, the AOC Champagne area has a very homogeneous geology, and this is reflected in Sr and Rb concentration.

Each sample of must was obtained from a single type of grapevine variety and from a small area of the AOC Champagne region, which covers the departments of Marne, Aisne and Aube. The results obtained for the concentration of inorganic elements in the musts analyzed by ICP-MS are shown in Table 4.

TABLE 4. Average and median of the elemental concentrations of 29 musts from the 2018 harvest.

N = 29	mg.L^{-1}							
	K	Ca	Mg	Na	B	Cu	Fe	Mn
Average	1,765	132	95	11	2.7	1.8	1.4	1.3
Median	1,655	129	89	10	2.7	1.9	1.2	1.2
SD	392	36	23	3	0.9	0.9	0.8	0.4
RSD %	22 %	28 %	24 %	32 %	33 %	51 %	56 %	34 %
N = 29	µg.L^{-1}							
	Al	Rb	Sr	Ba	Ni	Cr	Pb	Li
Average	725	495	270	61	40	12	8	1.5
Median	712	336	274	58	38	10	6	1.3
SD	367	338	87	25	14	6	5	0.9
RSD %	53 %	68 %	32 %	41 %	35 %	48 %	58 %	63 %

The concentrations of most elements in musts (Table 4) are in the same concentration range as those of Champagne (Table 3), except for Cu. For some elements, such as B, Na, Al, Cr, Mn, Fe, Sr and Ba, the concentration levels in the musts are similar to those recorded in the Champagne samples. However, for the recorded levels of some major elements, such as K and Ca, and trace elements, such as Cu, are much higher in the must than in the final Champagne; this can be explained by the precipitation of these elements during the winemaking process (Cheng and Liang, 2012). It must be noted that the RSDs obtained for all elements in the must are much higher than those recorded in the final corresponding Champagne products (between 2 and 10 times higher). The fact that the Champagne values are more homogeneous than the must values is likely a result of the two blending practices: first at must level and later at base wine level. It is worth mentioning that the average Pb content of must is similar to that of Champagne from the same year (2019). The low RSDs are attributable to the fact that all these musts are from the same year. It is not possible to directly link a specific must to a specific Champagne brand since this information is confidential and varies depending on the different brand producers. However, in terms of overall stability of the geology, in this study, the different concentration spans of the musts samples were compared into those of the different Champagne brands.

The relative dependence of Sr on Rb is plotted in Figure 4. The geographical origin of the 29 musts from the three main departments of the AOC Champagne region can be easily traced by their Sr and Rb content.

The Sr concentrations of the musts were generally in the same range as those of Champagne, ranging between 150 and 400 µg/L. In contrast, a much wider range of values was found for Rb content. In the musts from the Aube department, Rb levels ranged between 500 and 1300 µg/L, whereas in the Marne region musts, they ranged between 50 and 650 µg/L. The origins of the musts at local scale can be clearly discriminated in Figure 4. These findings are most likely due to the geology and different types and ages of bedrock from which the musts originate. The vast majority of the Aube vineyards are located on soil developed on upper Jurassic sediments (Limestone and Marl), differing from those in the Marne department where the vineyards are located on Tertiary bedrock (Limestone and Marl) or on upper Cretaceous bedrock (Chalk) (Vernhet, 2007). This can be linked to type of bedrock as mentioned previously (Gupta *et al.,* 2018). In the Marne department, the sediments are mostly chalk, while in the Aube department they are mostly chalk and dolomite.

Interestingly, the musts contain far different levels of inorganic elements compared to the final product, which is likely explained by the blending process. The musts originating from the Aube department represent only 15 % of total production in the Champagne region. To make Champagne, wines from the Marne, Aube and Aisne departments are blended. In a bottle of Champagne, Aube department base wine usually contributes to approximately 15 % of the total. Thus, the blending of the musts carried out during the Champagne-making process averages out the elemental concentrations of musts from different Champagne areas. None of the Champagnes analyzed in this study had a concentration of Rb higher than 400 µg/L, but all the Aube musts showed concentrations significantly higher than this value. The Champagnes studied here are mainly made from must produced in the Marne department. The grapes from the Aube department (where Pinot noir is the main variety) are mainly used to make red wines for the production of rosé Champagne.

It is worth noting that no differences were observed between the concentrations of elements in the musts from different grapevine varieties. Apparently, each grapevine variety accumulates the elements which it absorbs from the soil in similar concentrations. One rootstock (41B) is, however, so dominant in the Champagne production area that it was

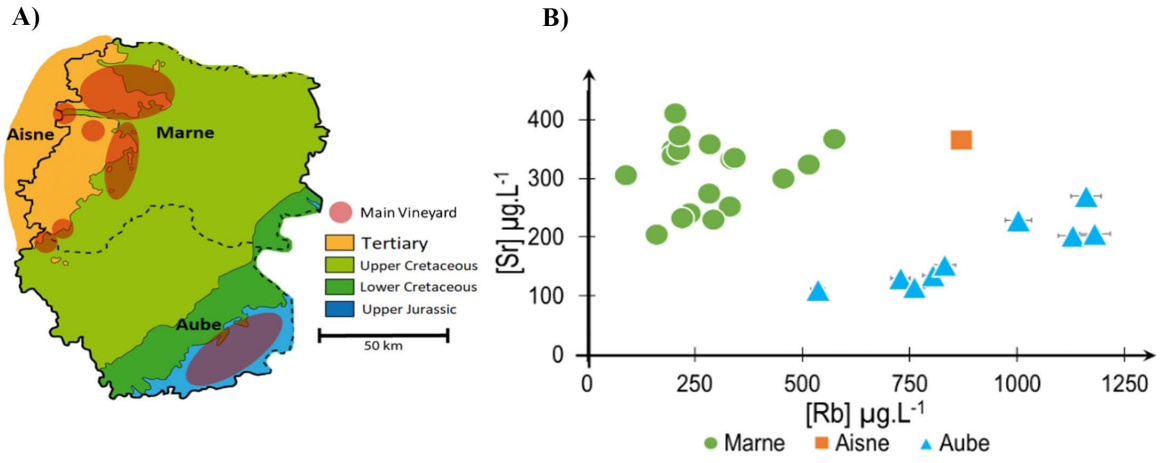


FIGURE 4. A) Location of the main vineyards of the champagne brands studied on a geological map of the region, and B). Sr and Rb concentrations of 29 musts from 3 different departments of the AOC Champagne region.

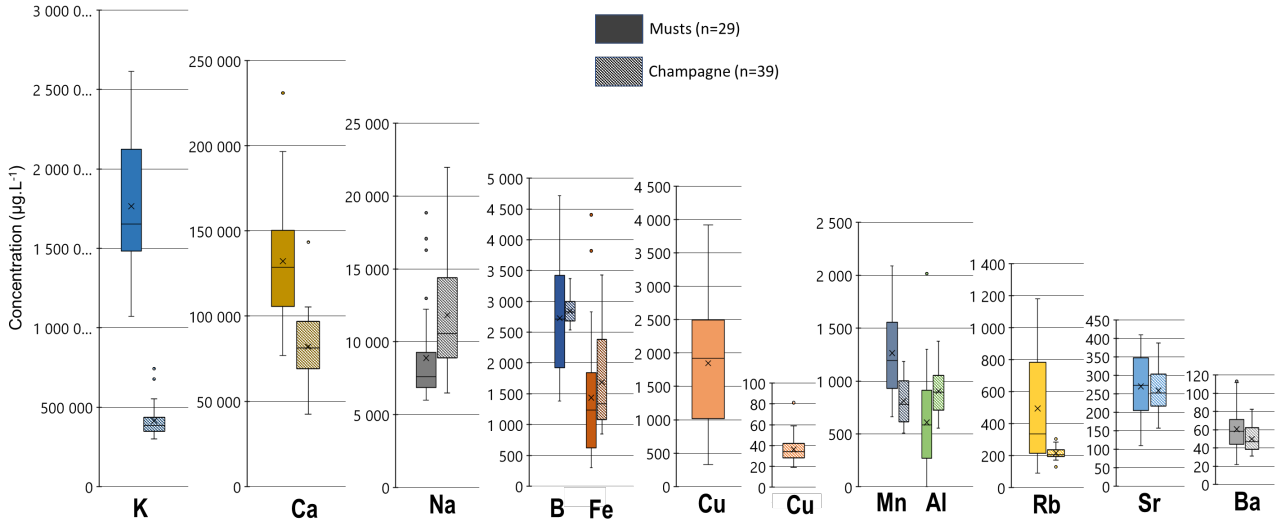


FIGURE 5. Concentrations of the inorganic elements K, Ca, Na, B, Fe, Cu, Mn, Al, Rb, Sr and Ba (µg/L) in 29 musts and 39 champagnes originating from the same AOC area presented as box-plots.
The elemental concentrations in the musts are represented by dark colored boxes and in the Champagnes by lighter colored boxes.

not possible to collect must samples from vineyards located on similar bedrocks which were grafted onto different rootstocks.

3. Blending: changes in elemental concentrations

To increase knowledge of the changes in inorganic multielemental signatures during the wine making process and the impact of blending musts compared to the final product, the multielemental signatures of the same 29 musts and 39 champagnes as previously discussed were compared (Figure 5).

The average concentrations of Rb, Sr, B, Ba, Mn, Fe, Al, Cr Na and Mg were similar throughout the Champagne-making process in terms of the median (Figure 5). The error bars likely decreased between the musts and the final Champagne as a result of the different blending steps which have an "averaging" effect. This tendency is clearly visible for Rb and B. The wider range of elemental concentrations in the musts is most likely related to geological variations throughout the area.

Some large changes were observed, however, in some of the analyses of the musts and the Champagnes. This was typically the case for K, which showed the largest drop in concentration between the musts and the Champagnes, with an overall decrease by a factor 5. This considerable decrease is mainly observed between the must and the base wine (from 3000 to 600 mg/L) and can be directly related to the winemaking process. The reduction in K by precipitation of potassium tartrate and potassium bitartrate is encouraged by winemakers (e.g., by cooling the wine) to avoid the formation of these precipitates in the bottle. Similarly, a decrease in Ca can also be observed, which can be explained by its precipitation as a salt of tartaric acid during the winemaking process. This calcium precipitation is not very intense, due to the high energy needed for calcium tartrate formation; it generally occurs on very old "*cuvées*", as it combines the effect of low temperature and low pH.

Another important element that showed very significant drops in concentration is Cu. A decrease in concentration of Cu by a factor of 60 can be observed between musts and Champagnes, which is also the direct result of the winemaking process. Vines are frequently treated with Cu (the spraying of copper is a common practice in vineyards to fight downy mildew or *Plasmopara viticola*), which explains high Cu concentrations in musts. Later in the process, within the reducing environment of the fermentations and in the presence of sulfur, a major fraction of Cu precipitates as CuS, which has a very low solubility. This precipitate is later physically separated from the resulting Champagne wine, which explains the very low Cu concentrations observed in the final product. The natural reduction of Cu is sought by winemakers, because the official limit of Cu in wine is 1 mg/L. These results are consistent with the work of Maria Goä Mez *et al*. (2004) who observed a similar decrease in Cu from must to wine.

All elements which precipitate in the tank during the winemaking process are removed by decanting; when the clean wine (supernatant) is transferred to another tank, the precipitates remain at the bottom of the tank. Overall, no minor or major elements, whether from the soil, air or treatment, increased during the winemaking process in the present study. Most elements are relatively stable, except for those displaying a significant drop in concentrations as described earlier for K, Ca and Cu. The production process, therefore, has no major effect on the elementary signatures for most of the elements considered.

4. Impacts of oenological products used in the winemaking process on the inorganic signatures of Champagne

During the Champagne production process, specific compounds are used as additives: yeast and sugar are added for both of the alcoholic fermentations, and bentonite (a specific type of purified clay) is added to clarify the wine. These products were analyzed to determine their influence on the isotopic signature of Champagnes during their production process.

4.1. Sugar addition

Sugar is the main additive in Champagne. During the "chaptalization" step (whereby the potential alcohol content of the must is increased), approximately 16 g/L of sugar is added and later during the second fermentation, known as "tirage", another 24 g/L is added. Finally, in the "disgorging" step, approximately 10 g/L is added. The multielemental signature of 1 g of sugar typically used for the winemaking processes was added. Based on a global average of 50 g/L of sugar addition to Champagne, the percentage provided by the sugar addition was calculated for 12 elements. The highest concentration was observed for Al, with a concentration of 350 ng/g in the raw product, and the lowest concentrations were observed for Li and Pb with 0.4 and 0.3 ng/g respectively. The contribution of Li and Pb represents less than 0.5 % of the total elemental concentration already present in the wine. However, the highest contributions were recorded for Ni, Cu, Fe and Al at 1.5, 1.4, 1.2 and 1 % respectively of the total elements in the Champagne. Regarding the other elements that are of interest as geological tracers, such as Sr, Rb, B and Pb, sugar additions also represented less than 0.4 % of the overall content of the concentrations of these elements in the final Champagnes. These results clearly highlight that, while sugar additions are common in the Champagne winemaking process, they do not influence the overall inorganic concentration of elements in the final products.

4.2. Yeast addition

Yeast is added to achieve the first and second alcoholic fermentations. The amount of dry yeast added for the fermentation step is around 100 mg/L (1.10^8 cells/mL). 1 g of yeasts was analyzed for their concentrations in all the study elements. High variations in elemental concentrations in the raw yeast were found depending on the element. The highest concentration was observed for Fe, with an average concentration of 73 µg/g. Pb had a much lower concentration of 2.6 ng/g); since only 100 mg/L of yeast is added to the wine, this only represents 1 10^{-5} g/mL and is negligible, even for elements with the highest concentration in the yeast. Yeast addition did not exceed 0.2 % of the total concentration of any of the elements. Hence, the addition of yeast does not influence the elemental signature of the final product.

4.3. Bentonite addition

Bentonite is an absorbent aluminum phyllosilicate clay, consisting mostly of montmorillonite. It is a highly absorbent natural clay containing silicate sheets and is widely used in winemaking. Exchangeable cations such as Ca, Mg, and Na are present in the interlayers of the clay. Proteins are depleted in wines due to their adsorption onto the surface of the silica layers of bentonite. Bentonite is added during the first fermentation and, even more importantly, during the tirage to prepare the second fermentation in the bottle. The amount of bentonite added during the first fermentation is lower than that added to the second fermentation, and it stays in contact with the wine for only one day.

TABLE 5. Elemental concentrations in two different types of bentonites.

µg.g^{-1}	Al	Fe	Ba	Mn	Sr	B
bentonite 1	51,079	4,101	3,084	83	53	40
bentonite 2	37,167	3,913	2,961	82	45	34
SD (n = 3)	9,625	827	627	10	5	3
µg.g^{-1}	Li	Rb	Cr	Ni	Cu	Pb
bentonite 1	12	7.8	2.6	1.9	1.7	1.3
bentonite 2	10	7.1	2.2	1.6	1.2	1.2
SD (n = 3)	1.3	0.8	0.5	0.1	0.1	0.5

The standard deviation has been calculated in triplicate on bentonite 1.

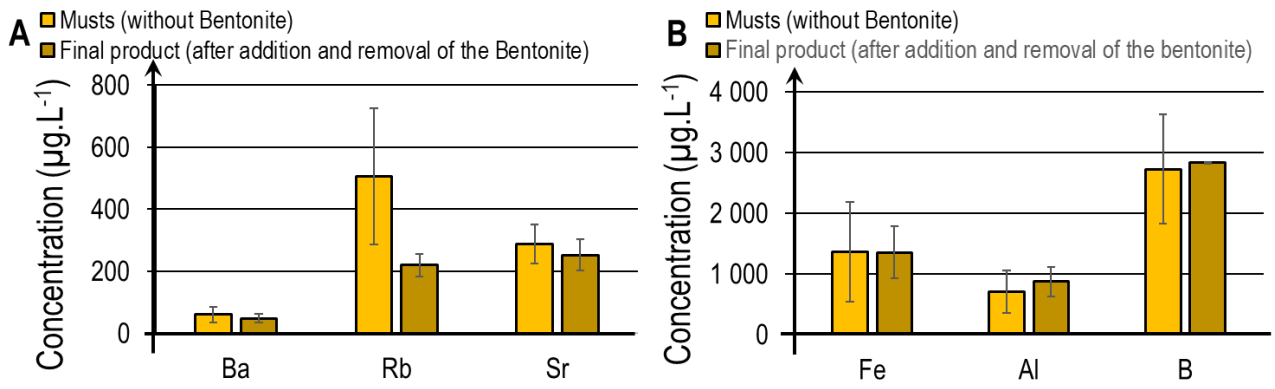


FIGURE 6. Concentrations of several elements (A: Ba, Rb and Sr) (B: Fe, Al and B) before (musts) and after the addition (champagne) of bentonite.

During the overall wine making process, a total amount of 240 mg/L of bentonite is generally added. Two bentonites (both being a mix of Na and Ca bentonites) traditionally used in the Champagne-making process were analyzed for their multi-elemental content. Sometimes, different relative percentages of the different bentonites are mixed; however, the exact procedures are not available. Therefore, in the present study both types of bentonites were analyzed separately in order to a obtain a general idea of their potential contribution to elemental content during the winemaking process. Similar concentration ranges for the elements were obtained for each of the bentonites analyzed (Table 5). The changes in concentration of some elements (Ba, Sr, Rb, Fe, Al and B) between the musts (before addition of bentonite) and the Champagnes (after addition and removal of the bentonite) can be seen in Figure 6. It appears that both bentonite 1 and 2 have the same overall effect on the content of inorganic elements in wines. The samples of musts originate from the 2018 harvest (same musts as previously described) and the Champagne from earlier years.

No significant differences in elemental concentration were found between the must before bentonite addition and the final product (Champagne), even after contact with the bentonite for several years. The same observation was made for the concentrations of other elements, such as Mn, Cr, Ni, Cu and Pb (data not shown). In general, it appears that there is little or no exchange between the bentonite and the final product or, if there is it cannot be detected. Regarding Ca and Mg (Tables 3 and 4), there is even a slight decrease in their concentrations after the bentonite treatment. One small exception is Li, which increases in concentration after the addition of bentonite. Catrino *et al*. (2008) showed that most of the inorganic elemental concentrations increase in still wine with the addition of bentonite, specifically Li. In the specific case of the Champagne-making process, bentonite is expulsed (with the yeast and other conglomerates) from the product during the disgorging step to obtain the final clear product, which may explain the differences between Champagnes and other still wines whose production process do not involve the expulsion of precipitates. In summary, the additives in the Champagne production process, including sugar, yeast and bentonites, do not alter the overall total elemental concentrations of Champagne, with the exception of Li.

CONCLUSION

In this study, six different brands of sparkling wines of Champagnes produced over the whole AOC area were analyzed by Q-ICPMS for their multielemental content. The elemental composition of both the musts produced in the different AOC areas and the final products were determined and compared. This study was performed for authentication and traceability purposes. The results show that the Champagnes were very homogeneous in terms of their inorganic concentrations, both in space and in time - some samples dating back to vintages as old as 1983. No significant differences in elemental composition were found between the six Champagne brands. More specifically, the concentrations of Sr and Rb were extremely homogeneous in all 39 Champagne samples. These results provide an opportunity for identifying potential false samples. The overall stability of concentrations of trace elements in Champagne, and more specifically the remarkable stability of Sr and Rb concentrations, results from i) the relatively homogeneous Champagne bedrock, and ii) the Champagne-making process, which involves several blending steps. Blending obviously has a role in the homogenization of multielemental signatures. The only element which displayed considerable changes over time was Pb, due to the decline in atmospheric contamination by Pb over the time span studied.

The analyzed musts can be directly linked to the impact of the bedrock. The elemental composition of must can be used to trace its origin within its production area. The department that the must originates from can be traced using Sr and Rb concentrations. The production process of Champagnes involves several blending steps, which contribute to averaging out the differences between elemental concentrations in musts, and therefore decrease the variability of these elements in Champagne.

The detailed study of the changes in trace element concentrations from must to the final product showed that, in general, no major changes to the elemental signature occur. Concentrations showed higher variability in the musts compared to the final products. Two elements, however, displayed a strong variation between the must and the final product. During the production process, a sharp decrease in concentrations of K, Ca and Cu was observed. These phenomena are normal and sought after by winemakers. K, Ca and Cu are precipitated during the production processes, and their precipitation is removed just prior to the final bottling step.

Additives used during the winemaking process were analyzed for their elemental concentrations. Main additives in the Champagne production process include sugar, yeast and bentonite. It appears that their addition does not significantly influence the overall elemental signature of the product, with the exception of Li, which increases. The other elements were either added in quantities that were too small or removed during the final step prior to bottling.

The analysis of elements throughout the Champagne-making process revealed that most of them were not influenced by the different steps involved. The elemental signature of the final Champagne is similar to the signature of the musts and therefore likely to be linked to the soil and bedrock underlying the vineyards. Moreover, blending does not modify the elementary signatures but homogenizes them, producing high stability in several elements (e.g. Sr, Rb, B). This information opens the door to future accurate authentication of Champagnes and the identification of counterfeits.

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