

## Characterization of the production regions of Chardonnay wines by analysis of free amino acids

by

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**S u m m a r y :** Free amino acids in 62 Chardonnay varietal wines, originating from Australia, Argentina, South Africa, Switzerland, United States and France (Pays d'Oc, Chablis, Pouilly-Fuissé and Côte de Beaune) were analyzed. They were differentiated according to the region of production using analysis of variance, Flash Table and Canonical Variate Analysis. Wines produced in the Pays d'Oc were identified by the presence of proline, while arginine and alanine served to identify Swiss wines. Chablis wines were characterized by 16 out of 20 major amino acids analyzed. Differences were correlated with climate and therefore with maturity at harvest. However, grape maturity alone did not explain the differences between wines. These differences were also correlated with the latitude of countries. Wines from Australia, Argentina and South Africa were not easily to differentiate. Côte de Beaune wines were distinguished from other Burgundy wines (Chablis, Pouilly-Fuissé), which can be explained by wine ageing on the lees after alcoholic fermentation for 4-6 months.

**K e y w o r d s :** free amino acids, wine, Chardonnay, Canonical Variate Analysis and Flash Table.

### Introduction

Ammonia and free amino acids are major nitrogen sources that can be assimilated and used by wine yeasts during fermentation. The must amino acid content is known to depend on the variety (FEUILLAT 1974; CANTAGREL *et al.* 1982; MILLERY *et al.* 1986; ETIEVANT *et al.* 1988; HUANG and OUGH 1991; SAUVAGE *et al.* 1993; PRESA-OWENS *et al.* 1995; SPAYD and ANDESEN-BAGGE 1996) but also on the geographic origin (OOGHE *et al.* 1981; SPAYD and ANDESEN-BAGGE 1996). In addition, vintage, climatic conditions (FLANZY and POUX 1965; FEUILLAT 1974; MILLERY *et al.* 1986; HUANG and OUGH 1991), degree of maturity (KLIEWER 1970, MILLERY *et al.* 1986) and duration of maceration (USSEGLIO-TOMASSET and BOSIA 1990; GUITART *et al.* 1997) have an effect on the amino acid fraction.

Important modifications of the amino acid fraction take place during alcoholic fermentation. Some amino acids are partially or totally metabolized by yeast cells during their growth phase when amino acids are used as carbon or nitrogen sources (FEUILLAT 1974; POLO and LLAGUNO 1974). Others will be released by yeast cells during the exorption phase (FEUILLAT 1980) or by proteolysis during the autolysis of dead yeasts (KLUBA *et al.* 1978; FEUILLAT and CHARPENTIER 1982; LURTON *et al.* 1989). Winemaking techniques, such as crushing (POUX 1968), addition of ammonium salts (FLANZY *et al.* 1962), must clarification (AYESTARAN *et al.* 1995) and yeast strain (OUGH *et al.* 1991; JIRANEK *et al.* 1995) also modify the amino acid composition.

Some authors have successfully used statistical methods to differentiate wine varieties by their amino acids (OOGHE *et al.* 1981; SYMONDS and CANTAGREL 1982; ETIEVANT *et al.* 1988; PRESA-OWENS *et al.* 1995; Akarepis 1997). In this study, amino acids were used to characterize the vintage and the different regions of origin.

This is the first attempt to compare 62 commercial Chardonnay wines from 9 viticultural sites in Europe, South Africa, USA, Argentina and Australia.

### Material and Methods

**S a m p l e s :** 62 Chardonnay wines were collected on occasion of the annual „Chardonnay du monde“ competition. The number of samples, the production area and vintages are shown in Tab. 1. All samples were bottled wines.

Table 1

List of wine samples

Code	Origin	Year					Total
		1990	1992	1993	1994	1995	
1	Pays d'Oc				3	4	7
2	Chablis	1	2	2	5	5	15
3	Pouilly-Fuissé			1	3	3	7
4	Cote de Beaune		2		4	2	8
5	Australia						5
6	Switzerland					5	5
7	Argentina						5
8	USA					5	5
9	South Africa					1	4

**A n a l y t i c a l m e t h o d s :** Ethanol, total acidity, pH and residual sugar were measured according to the methods of the Office International de la Vigne et du Vin (OIV 1990). For total nitrogen determination samples were subjected to Kjeldhal analysis.

**A m i n o a c i d a n a l y s i s :** The standard solution contained leucine (LEU), valine (VAL), phenylalanine (PHE),

aspartic acid (ASP), glutamic acid (GLU), lysine (LYS), isoleucine (ILEU), methionine (MET), tyrosine (TYR), glycine (GLY), histidine (HIS), alanine (ALA), arginine (ARG), serine (SER), threonine (THR) and proline (PRO) ( $2.5 \text{ mmol}\cdot\text{l}^{-1}$ ), cysteine (CYS) ( $1.25 \text{ mmol}\cdot\text{l}^{-1}$ ). An internal standard consisting of  $\alpha$ -amino-butyric acid (AabaSI) was added, along with asparagine (ASN), glutamine (GLN), ornithine (ORN) and  $\gamma$ -amino-butyric acid (GABA) ( $2.5 \text{ mmol}\cdot\text{l}^{-1}$ ).

**Derivatization procedure:** 200  $\mu\text{l}$  of wine, 40  $\mu\text{l}$  of internal standard amino acid,  $2.5 \text{ mmol}\cdot\text{l}^{-1}$  (AabaSI) and 760  $\mu\text{l}$  of milliQ water were added. 10  $\mu\text{l}$  of the above solution, 70  $\mu\text{l}$  borate buffer and 20  $\mu\text{l}$  of AQC reagent (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) were mixed for 12 s and placed for 10 min at  $55^\circ\text{C}$  (STRYDON and COHEN 1994). For liquid chromatography, two pumps (waters<sup>TM</sup> 626), a system controller (waters<sup>TM</sup> 600s) and an autosampler (waters<sup>TM</sup> 717 plus) were combined with a scanning fluorescence detector (waters<sup>TM</sup> 474).

Separation was carried out using a Nova-Pak<sup>TM</sup> C18 column (3.9 mm x 150 mm) filled with silica spheres (pore size: 6.0 nm; particle size: 4  $\mu\text{m}$ ). The precolumn had the same characteristics and was 2 cm long.

Eluant A was sodium acetate buffer, pH 5.8, eluant B was acetonitrile and eluant C was water. The gradient was:  $t=0$ : 100% A,  $t=35$  min: 86% A and 14% B,  $t=40$  min: 86% A and 14% B,  $t=51$  min: 0% A and 60% B and 40% C,  $t=54$  min: 100% A. Flow rate:  $1 \text{ ml}\cdot\text{min}^{-1}$ , column temperature:  $37^\circ\text{C}$ , detector wavelength: 395 nm.

**Statistical analysis:** Analysis of variance of the region effect was performed for each amino acid. The results were presented in a Flash Table (SCHLICH and DEGOUT 1995; SCHLICH 1998). The principle of this table is to gather amino acids in groups (separated by horizontal lines) of correlated variables and to compare each region mean to the grand mean. The table reports such a mean only when it is significantly (5%) higher (plus sign) or lower (minus sign) than the grand mean.

The regions were compared simultaneously for all amino acids using Multivariate Analysis of Variance (MANOVA) and a mapping of the regions was produced by Canonical Variate Analysis (CVA). The CVA was done by self-made SAS<sup>®</sup> macros. Basically, it is a well to the SAS<sup>®</sup> procedure

GLM using the MANOVA treatment and the CANONICAL option on this treatment. The variables are located on the canonical maps using their total correlation with the wine coordinates on these axes. On this map only the mean location of the region is represented. However, confidence (90% level) ellipses were drawn around these mean locations, not to the group membership, in order to assess region separation. This approach allowed to test the number of significant dimensions using a likelihood ratio test. These multivariate analyses were performed first across the 9 regions, then across the French regions and finally across the foreign regions except for Switzerland.

## Results

General parameters of Chardonnay varietal wines originating from different regions are shown in Tab. 2. Results of 21 amino acids are presented as mg amino acid per 100 mg amino nitrogen in order to minimize the variation in the nitrogen fraction arising from climatic conditions or viticultural practices. The regions of production will therefore be characterized as a function of the diversity of free amino acids in wine rather than by their quantitative values.

Flash analysis (Tab. 3) shows that all amino acids except asparagin, serine and glutamine are statistically significant. The central finding in this table is that the French wines are split from foreign ones because of their higher proportion in leucine, valine, phenylalanine and aspartic and glutamic acids. Pays d'Oc and Swiss wines are in a medium position; the proline content being higher in Pays d'Oc and lower in Swiss wines. We also note that wines from Argentina have a high proline content.

The MANOVA tests indicate highly significant differences for the 9 regions as well as for the 4 French regions analyzed separately. In contrast, this test was markedly less significant for the wines of the 4 other countries (Tab. 4).

The two first canonical components of the CVA of the wines originating from different regions (Fig. 1) yield 73.59% of the information. Wines from Switzerland can be clearly distinguished because of their high amounts of arginine and alanine and their smaller amounts of proline. Two groups can be differentiated. French wines, including Côte de

Table 2

pH, total acidity (mg l<sup>-1</sup> sulfuric acid), total nitrogen (mg l<sup>-1</sup>) and free amino acid nitrogen (mg l<sup>-1</sup> nitrogen) of wine samples from 9 origins

Origin	pH			total acidity			total nitrogen			free amino nitrogen		
	min	max	mean	min	max	mean	min	max	mean	min	max	mean
Pays d'Oc	3.11	3.64	3.46	2.8	4.6	3.50	175	280	230	100.2	205.7	154.7
Pouilly-Fuissé	3.32	3.52	3.44	3.3	4.1	3.57	217	287	253	131.5	193.2	161.7
Chablis	3.07	3.45	3.30	3.3	4.7	3.74	224	364	295	158.8	252.6	198.5
Cote de Beaune	3.14	3.38	3.32	3.5	4.8	4.20	231	413	314	174.7	276.9	241.4
Argentina	3.20	3.60	3.38	3.5	4.0	3.68	266	616	407	173.9	451.1	255.8
South Africa	3.31	3.61	3.41	3.7	5.1	4.33	364	784	567	237.1	532.8	370.9
Switzerland	3.50	3.57	3.55	3.3	4.0	3.64	434	686	564	317.6	476.7	387.8
USA	3.25	3.52	3.41	3.8	4.7	4.34	350	511	434	222.4	356.8	302.3
Australia	3.08	3.40	3.25	3.9	4.4	4.28	364	546	438	240.2	358.3	273.2

Table 3

Flash table for all Chardonnay wines

ATT	F <sup>a)</sup>	PRO <sup>b)</sup>	GMEA <sup>c)</sup>	Foreign					France			
				Argentina	Australia	S.Africa	USA	Switzerland	Pays d'Oc	C.Beaune	Chablis	P.Fuissé
LEU	22,8	0,000	0,152	0.077-	0.083-	0.099-	0.107-	0.110-		0.203+	0.206+	0.206+
VAL	24,4	0,000	0,101	0.049-	0.059-	0.078-	0.070-	0.113+		0.117+	0.133+	0.133+
PHE	15,3	0,000	0,073	0.033-	0.038-	0.047-	0.047-	0.087+		0.087+	0.098+	0.092+
ASP	21,2	0,000	0,116	0.053-	0.064-		0.067-			0.122+	0.165+	0.169+
GLU	19,3	0,000	0,167	0.111-		0.119-	0.102-			0.184+	0.209+	0.223+
LYS	8,35	0,000	0,176		0.089-	0.100-	0.099-			0.237+	0.237+	0.248+
ILEU	7,84	0,000	0,058	0.021-	0.024-		0.077+				0.073+	0.079+
MET	13,6	0,000	0,051	0.026-	0.024-			0.032-		0.080+	0.062+	0.062+
TYR	9,56	0,000	0,067	0.040-	0.048-					0.085+	0.083+	0.077+
CYS	7,56	0,000	0,051	0.025-						0.087+	0.060+	
GLY	9,23	0,000	0,125		0.092-	0.098-			0.099-	0.163+	0.144+	0.138+
HIS	3,23	0,005	0,048					0.036-			0.057+	
ASN	0,92	0,509	0,068									
ORN	3,15	0,005	0,057								0.081+	
GLN	1,62	0,140	0,016	0.002-								
ALA	6,07	0,000	0,399					0.691+	0.227-	0.298-		
ARG	10,3	0,000	0,193			0.326+		0.417+	0.100-	0.124-	0.148-	0.074-
GAB	2,55	0,020	0,205		0.341+				0.095-			
SER	1,63	0,137	0,114					0.206+				
THR	6,21	0,000	0,080	0.055-				0.124+	0.049-		0.094+	
PRO	6,91	0,000	3,827	4.607+				2.742-	4.871+		3.481-	

<sup>a)</sup> Fisher level of the region effect. 8 and 51 degrees of freedom. <sup>b)</sup> Level of significance. <sup>c)</sup> Grand mean of amino acid.  
+, -: see Materials and Methods.

Beaune, Chablis, Pays d'Oc and Pouilly-Fuissé, show a high proportion of a large number of amino acids and a small proportion of alanine, arginine and  $\alpha$ -amino-butyric acid. The other group of foreign wines shows a smaller proportion in a large number of amino acids.

As demonstrated by the likelihood ratio test, 4 axes were significant (Tab. 4). However, plotting axis 3 against axis 4 indicates only the separation of Pays d'Oc due to the higher level of proline.

In the CVA of the French wines two axes are significant (Fig. 2). The first axis shows two clusters, one consisting of Pays d'Oc wines, characterized by proline as stated above, the other consisting of wines from Chablis and Pouilly-Fuissé being positively correlated with a large number of different amino acids. Along the second axis wines from the Côte de Beaune are grouped.

The CVA of foreign wines is performed with the 10 most discriminant amino acids, since the number of samples is too small compared to the number of variables. Only one axis was significant in distinguishing wines from the United States from the others due to their higher proportion in iso-leucine and methionine.

### Discussion

Various studies have demonstrated a sharp increase in proline at the onset of maturation (KLIWER 1970; MILLERY *et al.* 1986). Proline is usually not metabolized by yeasts

Table 4

MANOVA results for Chardonnay wines

	MANOVA F <sup>a)</sup>	p <sup>b)</sup>	Significant different axes at 10% level <sup>c)</sup>
9 wines; all origins	4.43	0.0001	4
4 French wines	3.07	0.0003	2
4 foreign wines (10 amino acids; $\alpha < 25\%$ )	2.1	0.0547	1

<sup>a)</sup> Approximation of Hotelling-Lawley trace.

<sup>b)</sup> Level of significance.

<sup>c)</sup> Likelihood ratio test.

during the alcoholic fermentation in nitrogen rich musts like those from Chardonnay grapes. It can serve as an indicator in the wine for the degree of maturity attained by the grape.

The significant differences observed for proline between wines of the Pays d'Oc, from Switzerland and Chablis might be explained by the differences in climate and therefore in maturity at harvest. If this is true, countries with a hot climate like South Africa or Australia should yield higher proline levels. This is the case for Argentinian wines but not for Australian and South African wines.

Total acidity and pH are indicative parameters of the ideal date of grape harvest. Furthermore, according to regulations, tartaric acid can be added to must in Europe and the

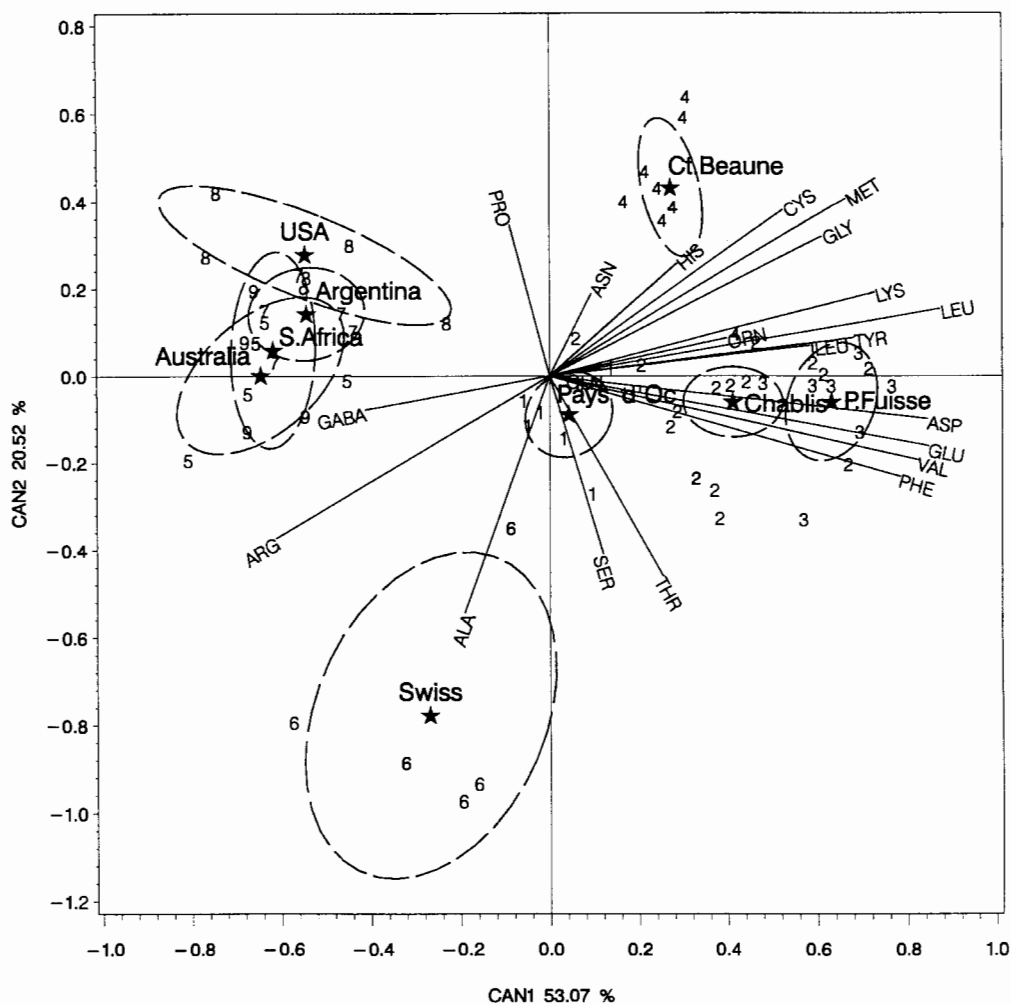


Fig 1: Canonical Variate Analysis plot of the 9 origins (codes refer to Tab. 1).

addition of malic acid to must and wine is permitted in the USA, Argentina and Australia. This is a common procedure in hot countries to raise the acidity of must. Therefore, pH and total acidity values cannot be used to estimate the degree of maturity.

Studies by FEULLAT (1974) and SPONHOLZ (1991) showed that it is possible to correlate must nitrogen levels with maturity. Grapes originating from southern latitudes generally have less nitrogen than those from northern regions.

Tab. 4 shows that wines from South Africa, Australia, the United States and Argentina, have greater quantities of total nitrogen and free amino acids than French wines. These countries may harvest before full maturity is reached to maintain higher acidity. Furthermore, the addition of ammonium salts was rather common in southern countries like Australia and South Africa (JIRANEK *et al.* 1995).

Grape maturity alone does not explain the differences in wine. For example, we observed a marked divergence for Swiss wines, despite having total nitrogen levels similar to those of South Africa. On the other hand, wines from the southern hemisphere are poorly distinguishable (Figs. 1 and 3). This is in agreement with studies by ETIEVANT (1988) and KAUFMAN (1997) who attributed similar differences in wines to the latitude of the production zone.

French wines (except of Pays d'Oc), which originated all from Burgundy, were characterized by a larger number of amino acids (LEU, VAL, PHE, ASP, GLU, LYS, ILEU, MET, TYR, CYS and GLY) compared to foreign wines.

In the present findings we did not observe a vintage effect. Chablis wines, for instance, consistently group in the same zone regardless of the year (Fig. 3). This agrees with results of PRESA-OWENS (1995), who distinguished between wineries, and therefore winemaking practices, but not with results of SEEBER *et al.* (1991) who evidenced the contribution of the amino acids to the discrimination of Chardonnay wine from a relatively small geographical area of Trentino (Italy), if at least two vintages were considered.

However, great care must be taken when commercial wines are used because these wines are not standardized products.

Wines from Côte de Beaune were significantly different from other French wines (Figs. 1 and 2). They are characterized by a specific oenological practice, i.e. the ageing of wine on the lees for 4-6 months after alcoholic fermentation. FERRARI and FEULLAT (1988) have demonstrated that in this period nitrogen compounds and in particular amino acids are released. This is due to the release of cell vacuolar contents and yeast autolysis (FEULLAT and CHARPENTIER 1982)

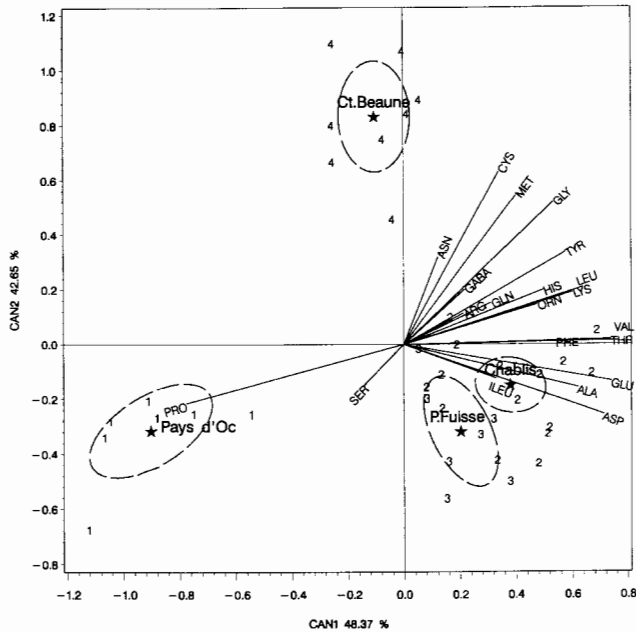


Fig 2: Canonical Variate Analysis plot of the French wines (codes refer to Tab. 1).

by a proteolytic activity (LURTON *et al.* 1989). For Côte de Beaune wines this ageing on the lees is evidenced by a higher proportion of amino acids, 77 % of total nitrogen, compared with 65 % in other French wines. This is in agreement with studies of LURTON *et al.* (1989) which demonstrate the release of all amino acids except threonine, arginine and histidine during autolysis. It explains why no change in the amino acid profile of the Côte de Beaune compared to other French wines can be detected (Tab. 3).

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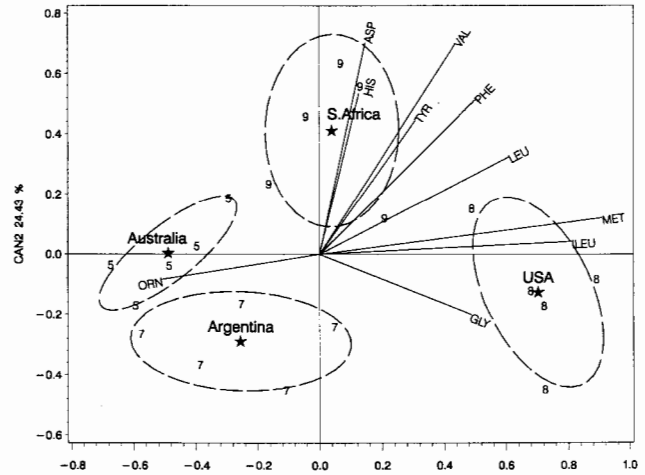


Fig 3: Canonical Variate Analysis plot of the foreign wines except Swiss ones (codes refer to Tab. 1).

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