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Effects of maceration on the amino acid content of Chardonnay musts and wines

by

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S u m m a r y : Chardonnay musts were macerated for 0, 6, 12, and 18 h and wines analyzed immediately after fermentation and at 6 months of bottle ageing. Maceration implies an increase of the amino acid content in the must immediately after fermentation and in the final wine. Wines from macerated musts have significantly higher levels of γ-amino butiric acid, serine, glycine, histidine and alanine than wines from non-macerated musts. The content of these amino acids could help to examine whether there has been a maceration process or not.

Must macerated for 6 h is characterized by higher contents of almost all amino acids. The only exceptions were glycine and glutamine. It is concluded that the optimum maceration time for Chardonnay must is 6 h when a maximum amino acid content is reached. After 6 months the amino acid concentrations in bottled wines were higher than in wines shortly after fermentation. Obviously the final equilibrium of amino acids had not been reached and during bottle ageing amino acids continued to be set free into the medium from yeast cells or their autolysis. During this time wines tend to reach a similar amino acid concentration independent of the maceration time of their respective musts.

K e y w o r d s : PITC amino acids, must, wine, maceration, Chardonnay, HPLC, Principal Component Analysis.

Introduction

While the importance of maceration for the production of red quality wine is well established it seems that maceration in the production of quality wines from white grapes is not common.

Maceration implies significant modification of wine composition: decrease of acidity, increase of color and phenolic substances. According to OUGH (1969) the wine quality is impaired when maceration takes place for more than 12 h. The sensorial improvement due to maceration seems to be modest and is not attained with all grape varieties (CASTINO *et al.* 1990).

With Chardonnay it is common practice to macerate the must for a short time to favour varietal aroma extraction. BARILLÈRE *et al.* (1990) stated that organoleptic characteristics of Chardonnay wines from Limoux are improved by maceration which reduces acidity and gives more aroma to the final wine. However, they conclude that this does not hold true for all varieties.

According to DEFRANOUX and JOSEPH (1992) maceration leads to positive results in Chardonnay if complete maturity is not reached at harvest. They propose an optimum maceration time of ca. 15 h.

According to ARNOLD and NOBLE (1979) maceration of Chardonnay must can increase aroma quality and wine structure without increasing astringency. They recommend a maceration time of 16 h, while HAUSHOFFER (1978) recommends maceration times of less than 5 h.

DUBOURDIEU *et al.* (1986) carried out different experiments with maceration times of 8 to 18 h at temperatures between 18 and 22 °C. Skin contact increased the amino acid content of the must.

Given that amino acids will be yeast nutrients during the fermentation process, and that a stuck fermentation will cause important problems, we considered to carry out a quantitative study of the amino acid content of macerated Chardonnay musts and wines.

Material and methods

A m i n o a c i d a n a l y s i s : A liquid chromatograph (KONTRON system 400) fitted with two alternating twin piston pumps (model 420), a high pressure mixing chamber (model M-491), an automatic sample injector (model 460), a thermostat-controlled column oven (model 480), and a KONTRON UV-Vis detector (model 430) of variable wavelength were used. Separation was carried out using a VYDAC C18 column (250 mm x 4.6 mm i.d.), filled with silica beads with a pore size of 30 nm and a particle size of 5 µm. The precolumn used had exactly the same characteristics and was 3 cm long.

A standard 2.5 mM amino acid solution containing aspartic acid, glutamic acid, serine, glycine, threonine, alanine, histidine, proline, arginine, valine, tyrosine, methionine, isoleucine, leucine, phenylalanine, and lysine was supplied with phenylisothiocyanate (PITC). Individual amino acids of the standard plus α-amino butyric acid, γ-amino butyric acid and glutamine were supplied by Sigma Chemicals, ammonium acetate by Merck, ethanol and triethylamine (TEA) by Scharlau, S.A., acetonitrile and methanol by Romil Chemicals. The water used to prepare the solutions was purified by a Milli-Q (Millipore) system. The coupling buffer used for derivatization was made of acetonitrile, ethanol, triethylamine and water, (10:5:2:3,

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v:v:v:v). The mobile phase was prepared daily. The total flow rate was 2 ml min^{-1}. Solvent A: 50 mM ammonium acetate buffer, pH = 6.5; solvent B: 100 mM ammonium acetate buffer, pH = 6.5 in acetonitrile:water 1:1. Solution pH was adjusted to 6.5 with acetic acid. All liquids were filtered through a membrane filter with 0.45 µm average pore size (Millipore) prior to use.

200 µl of sample plus 50 µl of internal standard (2.5 mM α-amino butyric acid, α-ABA) were put in a 10 ml test tube. After drying by vacuum and resuspending the residue in 100 µl of coupling buffer the solution was dried again. 100 µl of coupling buffer and 5 µl of PITC were added and left at room temperature for 20 min. After drying the residue contained the PITC amino acids which were separated with the following linear gradient: from min 0 to min 45 the gradient increased from 0 to 70 % of solvent B. It was kept for 1 min at 70 %, reaching 100 % of solvent B by min 48. The entire gradient cycle lasts 60 min, including the time for stabilizing the column after each injection. Column temperature is 50 °C and detections are performed at 254 nm. The analysis takes 28 min.

The method for amino acids analysis has been described by GUITART *et al.* (1996).

Sample preparation: Four homogeneous lots of Chardonnay grapes from Somontano in Spain were machine-harvested, destemmed, crushed, and sulphite was added (5 g/hl). Three of the lots were macerated in duplicate for 6, 12, and 18 h at 18 °C in six FABRI macerators (25,000 kg capacity) and refrigerated at 4 °C. The fourth lot was not macerated; it was pressed in a pneumatic press at 2 kg maximum pressure. After pressing, all lots were centrifuged and racked to 20,000 l Stork stain steel deposits. A starting culture was added to each deposit. Commercial yeast ("fermiblanc Arom", *Saccharomyces cerevisiae* SM102) was inoculated. Yeast was prepared in warm sugared water, adding 10 g per hl of must, which means about 25 x 10^{10} yeast cells per hl. Fermentation took place at 14 °C. After 15 d of fermentation, wines were racked and a sample was taken for analysis. Subsequently the wines were bottled without filtering and a sample was taken again for analysis 6 months later. Wines were tested by six experienced wine judges, using the triangular test (IRN, UNE 87-006). In all cases an analysis was performed with a 200 µl ultrafiltered aliquote of the sample plus 50 µl of an internal standard (α-ABA). After derivatization the residue was suspended in 1 ml of chromatographic aqueous solvent (Solvent A) and 80 µl were injected in the HPLC system. All samples were analyzed 3 times.

Analysis of other enological parameters: The musts and wines obtained were analyzed for total acidity, tartaric acid, pH, total soluble solids, degree alcohol, potassium, volatile acidity and sugars according to the AOAC methods (AOAC, 1970, Tab. 1).

Statistic analysis: NTSYS 1.7 (ROHLF 1991) was used. Principal Component Analysis was performed on samples (musts and wines) and on variables (amino acids).

Table 1

Analysis of must and wine constituents after different times of maceration

	Maceration time (h)	°Brix	Alcohol (% v/v)	pH	Titratable acidity (g/l sulph.)	Tartaric acid (g/l)	K (mg/l)	Volatile acidity (g/l)	Sugar (g/l)
Must	0	21.2		3.36	4.63	5.93	1672		
	6	20.8		3.34	4.77	5.58	1729		
	12	20.2		3.29	4.99	5.24	1716		
	18	20.2		3.33	4.81	5.00	1740		
Wine	0		12.2	3.31	4.73	2.45	902	0.22	1.10
	16		12.2	3.31	4.92	2.60	1066	0.27	1.10
	12		11.8	3.18	4.88	2.50	950	0.22	0.95
	18		11.7	3.26	4.99	2.52	1014	0.22	1.20

Results and discussion

Musts: The amino acid content has been determined in musts macerated for 0 to 18 h (Tab. 2). The values for arginine and alanine after 6 and 18 h of maceration are not presented, as the chromatographic peaks overlapped and therefore could not be quantitified. If we consider the sum of both peaks as arginine, its quantity in must is 291.1 mg/l (6 h maceration), and 309.5 mg/l (18 h).

Obviously proline is the main amino acid in Chardonnay grape must: 870 and 1771 mg/l, depending on must maceration duration. Proline is 4 times higher than the other main amino acids alanine, arginine, and glutamine; the minor amino acids are tyrosine, valine, glycine, isoleucine, leucine and phenylalanine. Lysine was not found in any of the musts.

These results agree with those of HUANG and OUGH (1991), who carried out a quantitative analysis of amino acids in musts and wines of Chardonnay.

After maceration musts had a higher amino acid content with the exception of aspartic acid, proline, and methionine which have lower concentrations after 12 h of maceration.

Table 2

The amino acid content of Chardonnay musts (mg/l) macerated for 0, 6, 12 or 18 h

Amino acids	Maceration time (h)			
	0	6	12	18
Asp	39.5	50.4	31.4	46.5
Glu	59.7	73.4	67.2	69.5
Ser	93.2	138.7	107.1	134.6
Gly	12.7	18.5	16.2	21.1
Gln	194.6	278.1	278.0	300.0
His	58.9	124.1	73.6	103.9
Gaba	57.4	82.3	64.1	81.2
Thr	50.6	90.5	54.8	83.0
Ala	104.3	291.1*	102.9	309.5*
Arg	101.4		227.8	
Pro	1146.5	1771.6	870.4	1380.6
Tyr	17.1	29.9	24.2	27.1
Val	33.0	49.6	34.3	43.7
Met	12.9	17.2	9.9	15.7
Ile	11.1	20.3	14.2	18.5
Leu	14.9	28.2	20.0	24.3
Phe	13.6	28.1	17.2	24.0
Lys	0.0	0.0	0.0	0.0
Total	2021.4	3092.0	2013.3	2683.1

* Sum of arginine (Arg) and alanine (Ala)

After 6 h of maceration all amino acids are at their maximum, except for glycine and glutamine which reach a maximum after 18 h. Thus, the optimum maceration time of musts, i.e., the time at which the amino acid content is at its maximum, is 6 h. Moreover, wines from musts with a maceration time of 6 h were preferred in a wine tasting.

Changes in the concentration of amino acids can be summarized as follows: an increase in concentration was observed from non-macerated to 6-h-macerated musts, thereafter this level was maintained or decreased (18 h maceration). Musts macerated for 12 h had lower levels than those macerated for 6 and 18 h.

The amino acid data were analyzed statistically by Principal Component Analysis to establish similarities and differences of behavior with respect to maceration. Results of Fig. 1 account for 100 % of the variance. As expected, the non-macerated must (MMC0h) is most different from the other three, and it is the only one with positive values in the first (x-axis) and second component (y-axis).

Must from 6-h-maceration (MMC6h) differs from the rest in its valine, threonine, histidine, phenylalanine, γ-amino butyric acid, isoleucine, glutamic acid, serine, leucine, and tyrosine contents. The sample macerated for 18 h shows higher levels of glycine and glutamine, even though such variables affect in the same manner the 6-h-sample; in both samples the levels are very similar.

Methionine, proline and aspartic acid are grouped in the upper left corner of the diagram, being negatively correlated with the 12-h-sample. These amino acids have a similar behavior, and show a minimum level at 12 h of maceration. They reach a maximum at 6 h.

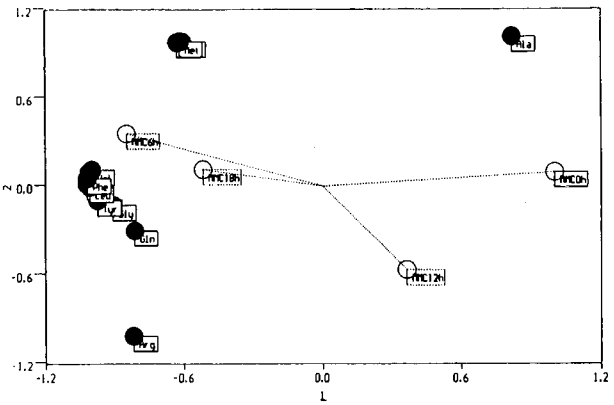

Fig. 1: Projection of musts and amino acids on Principal Components 1 (x-axis) and 2 (y-axis). Musts underwent different maceration times: 0 h = MMC0h; 6 h = MMC6h; 12 h = MMC12h; 18 h = MMC18h. Amino acids: Asp, Glu, Ser, Gly, Gln, His, Gaba, Thr, Ala, Arg, Pro, Tyr, Val, Met, Ile, Leu, Phe, Lys.

Alanine and arginine were determined only in the 0-h- and 12-h-samples, and that is why they occupy strategic positions in the figure.

Wines immediately after fermentation: The amino acid content of the 4 wines obtained from non-macerated and macerated musts is shown in Tab. 3. Threonine is absent in the analysis due to the fact that the initial amount of this amino acid in must disappears during alcoholic fermentation. However, there is a small amount of lysine in these wines, which was absent in must.

As expected, maceration of must caused an increase in the amino acid content of the wine, similarly to that

Table 3

The amino acid content of wines (mg/l) from non-macerated and macerated musts shortly after fermentation

Amino acids	Maceration time (h)			
	0	6	12	18
Asp	3.8	5.4	3.1	3.2
Glu	13.7	24.0	20.8	18.5
Ser	4.8	7.5	9.0	9.8
Gly	16.8	29.0	31.6	31.4
Gln	18.6	18.8	25.8	27.8
His	9.2	9.2	11.6	10.8
Gaba	87.8	112.2	105.3	103.5
Thr	0.0	0.0	0.0	0.0
Ala	13.9	38.4	84.0	61.5
Arg	55.9	88.1	71.0	66.8
Pro	1001.7	992.4	920.9	910.6
Tyr	2.7	3.8	4.8	3.7
Val	2.9	3.4	3.1	2.9
Met	20.1	22.2	23.9	26.9
Ile	2.1	2.4	1.0	2.3
Leu	3.1	8.1	2.3	6.8
Phe	2.6	3.4	2.2	3.9
Lys	3.3	6.8	5.0	4.8
Total	1263.0	1375.1	1325.4	1295.2

which occurred in must. The only exception is proline which shows the same concentration in wines from non-macerated musts and those from a 6-h-macerated must. Proline which slightly decreased with longer maceration times is the main amino acid in musts and wines from Chardonnay. It behaves unusually and is clearly different from the other amino acids.

The influence of maceration on the amino acid content of wines is due more to the maceration process than to the maceration time. The amino acid content in wines from 12- and 18-h-macerated musts is very similar to that of most amino acids.

The variation of amino acids in wines due to maceration cannot be assigned to a single generalized pattern. We have to take into account that amino acids are metabolized by yeasts during fermentation, but not all of them to the same extent. Therefore, the presence of a given amino acid in the must together with the rest of the nitrogen compounds, may be responsible for the final amount of another amino acid in the final wine.

Some amino acids such as serine or glycine, increased in wines with increasing maceration time reaching a maximum level in wines from 12-h- and 18-h-macerated musts. Glutamine was similar in wines from 0 and 6 h; it increased in wines from 12-h-macerated must, and remained at that level in wines from 18-h-macerated must. Histidine, alanine, and tyrosine reached a maximum in wine after 12 h maceration, while phenylalanine was at its maximum after a maceration of 18 h.

With the exception of these 7 amino acids and proline, the rest of the amino acids reached a maximum in the wine after 6 h of maceration.

Fig. 2 shows the Principal Component Analysis, projecting samples and variables on the plane formed by the first and second components which account for 84 % of the total variance.

Wines from non-macerated musts appear to be more clearly separated from the other wines. Wines from macerated musts occupy the right hand side of the figure. The first component (x-axis) shows the separation of wines by hours of must maceration; it accounts for 47 % of the variance. The abscissa is negative for the wine from the non-macerated must, while it is positive for wines from macerated musts. The second component (y-axis) differentiates the wine from 6-h-maceration which is the only one with a positive contribution to that component.

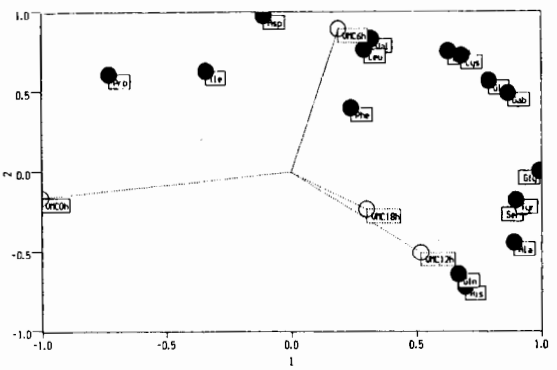

Fig. 2: Projection of wines analyzed immediately after the end of fermentation and their amino acids on Principal Components 1 (x-axis) and 2 (y-axis). Wines originated from musts which underwent different maceration times: 0 h = VMC0h; 6 h = VMC6h; 12 h = VMC12h; 18 h = VMC18 h. Amino acids: see Fig. 1.

Thus, the general effect of must maceration on the final wine is an increase of all amino acids except proline.

Wines from musts macerated for 12 and 18 h appeared to be grouped and, therefore show a similar behavior. They are characterized by the presence of amino acids such as glutamine, histidine, alanine, serine, tyrosine and glycine. Isoleucine and aspartic acid, being positioned in the opposite corner of the diagram, are negatively correlated and have lower levels.

The wine from 6-h-macerated must (VMC6h) is characterized by a higher level of isoleucine, aspartic acid, valine, leucine, lysine, glutamic acid, γ-amino butyric acid and arginine.

Phenylalanine was similar in wines produced from musts which were macerated for 6 and 18 h; it has an intermediate position between both samples.

Wines at six months of bottle ageing: Finished wines were racked and 2 g/hl of SO_2 added before bottling to prevent microbial activity which might influence the amino acid composition of the wines. Six months later the wines were analyzed to study the effect of bottle storage.

The amino acid content of the wines stored for 6 months indicates an increase of all amino acids during that time (Tab. 3 and 4) except for glutamic acid and proline which remained constant during that period independently of maceration time.

Similar observations were made by FERRARI and HORY (1988) with Chardonnay wines. These authors observed

Table 4

The amino acid content of wines (mg/l) from non-macerated and macerated musts at 6 months of bottle ageing

Amino acids	Maceration time (h)			
	0	6	12	18
Asp	10.2	12.8	10.9	10.3
Glu	16.4	24.5	23.8	18.7
Ser	8.1	11.4	10.7	10.5
Gly	21.8	32.8	35.2	34.6
Gln	1.9	2.1	2.5	2.6
His	41.2	42.0	43.0	42.2
Gaba	91.1	119.5	112.1	114.8
Thr	13.1	9.7	8.4	8.8
Ala	18.9	45.8	88.7	64.6
Arg	57.9	92.8	77.9	70.3
Pro	1007.5	1003.9	935.0	947.1
Tyr	10.2	8.5	9.3	7.9
Val	8.5	9.0	8.1	5.4
Met	74.0	35.4	44.3	39.5
Ile	4.0	4.7	4.2	4.0
Leu	18.1	24.4	20.5	20.4
Phe	4.4	5.6	5.0	4.6
Lys	12.9	14.7	13.2	12.6
Total	1420.2	1499.6	1452.8	1418.9

that certain amino acids remained stable during aging while others such as lysine, leucine, glycine and valine increased. In our case, histidine showed a higher increase.

It is ineresting to note that threonine which had disappeared shortly after fermentation was detected again 6 months after bottling.

It can be assumed that the final equilibrium was not yet reached after six months and that amino acids have been set free probably from proteins.

Again, the amino acid concentration is higher in wines from macerated compared to non-macerated musts, but the differences are less pronounced than in wines immediately after fermentation. Amino acids such as histidine, isoleucine and phenylalanine remained constant, and their concentration was independent of maceration time.

Threonine, tyrosine, proline and methionine showed higher levels after 6 months in wines from non-macerated compared to macerated musts.

In wines from must macerated for 6 h the other amino acids were at their maximum concentration, but this was not very different from that of wines originating from musts with 12 or 18 h macerating. Alanine is the only amino acid that increased up to 12 h of maceration and decreased slightly after 18 h of maceration.

It is concluded that after six months wines tend to be similar in their amino acid content, independently of maceration time of the musts.

Principal Component Analysis of these results is shown in Fig. 3. First and second components account for 92 % of the total variance. The first component (x-axis) separates wines as a function of must maceration time. Wine from non-macerated must presents a positive value, while wines from macerated musts have a negative value. Similar to wines shortly after fermentation, wines after 6 months of storage from musts with longer maceration times are more similar than wines from non-macerated or 6-h-macerated musts.

Wine from a non-macerated must has a higher content of methionine, threonine and tyrosine. Proline is situated between the non-macerated and the 6-h-macerated wine, therefore its contribution to these two samples is almost the same. It was negatively correlated with wines from musts macerated for 12 and 18 h.

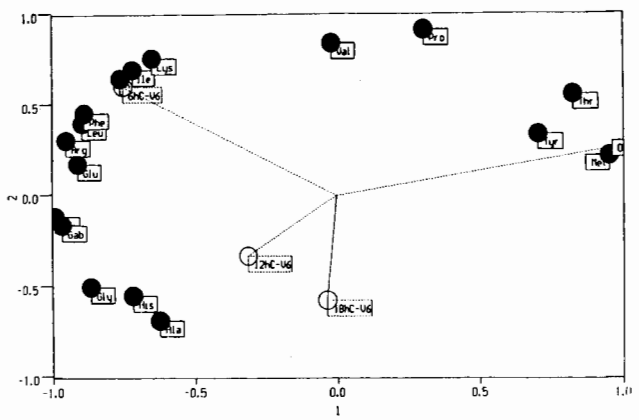

Fig. 3: Projection of wines analyzed after 6 months of bottle ageing and their amino acids on Principal Components 1 (x-axis) and 2 (y-axis). Wines originated from musts which underwent different maceration times: 0 h = 0hC-V6; 6 h 6hC-V6; 12 h = 12hC-V6; 18 h = 18hC-V6. Amino acids: see Fig. 1.

Wines from musts which had been macerated for 6, 12 or 18 h have significantly higher levels of γ–amino butyric acid, serine, glycine, glutamic acid and alanine than wines from non-macerated musts. We assume that the content of these amino acids in the samples indicate whether or not there has been a maceration process.

After 6 months the wine sample from 6-h-macerated must accumulates a higher number of variables. Glutamic acid, arginine, leucine, phenylalanine, aspartic acid, isoleucine and lysine show positive values in the second component, and contribute negatively to the first component which has a value similar to the sample.

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