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Status of yeast assimilable nitrogen in Italian grape musts and effects of variety, ripening and vintage

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Summary

The content of promptly assimilable nitrogen by yeast (PAN) was analysed in 586 juices from technologically ripe grapes collected in several Italian regions. A spectrophotometric method within the reach of each wine laboratory was used, adapting a previous method of the American Society of Brewing Chemists used for wort to grape juices. 58.3 % of the samples were below the classic deficiency threshold of 140 mg·l^{-1}. Among varieties and vintages there were significant differences. An overall trend of PAN to slightly decrease with ripening was observed. The variability of the PAN content of numerous samples harvested in a vineyard to check ripeness seems to be larger than that for sugars and total acidity.

K e y   w o r d s : yeast assimilable nitrogen, grape variety, grape ripeness, vintage, Italian regions, nutritional status.

Introduction

Among the nitrogen sources available for yeast in grape juice, ammonium and free amino acids are definitely the most important. They promote cell growth of yeast and affect significantly both the winemaking process (*e.g.* fermentation rate, alcohol yield, residual sugars (Bisson 1991; Kunkee 1991) and the sensory quality of wine, the latter mainly through the final volatile products of the fermentation metabolism of yeasts. In fact, the availability of the cited nitrogen sources favours, to different extents, the production of acetates of higher alcohols and ethyl esters of fatty acids by yeast (Ough and Lee 1981; Rapp and Versini 1991, Versini *et al.* 1992; Bosso 1996; Nicolini *et al.* 1996, 2000) and limits that of higher alcohols, *e.g.* isoamyl alcohols and 2-phenylethanol (Äyräpää 1971; Ough and Bell 1980; Houtman and Du Plessis 1981; Margheri *et al.* 1984; Rapp and Versini 1991; Seeber *et al.* 1991; Bosso 1996; Nicolini *et al.* 1996). Moreover, it limits the production of sulphur-containing compounds liable for several off-flavours (Vos and Gray 1979; Monk 1982; Margheri *et al.* 1984; Tromp 1984; Henschke and Jiranek 1991; Seeber *et al.* 1991; Bosso 1996; Jiraneck 1999; Nicolini *et al.* 2002). Among the amino acids, however, *Saccharomyces* does not metabolise proline under normal conditions of fermentation, since oxygen is necessary for this (Boulton *et al.* 1996). From this proposition the concept of promptly assimilable nitrogen (PAN) is derived which, even if under different acronyms, is always expressed as the sum of nitrogen present as ammonium and amino acids, proline excluded (Ingledew and Kunkee 1985; Bely *et al.* 1991; De Iure 1995; Sablayrolles 1995; Dukes and Butzke 1998). Several authors defined an assimilable nitrogen deficiency threshold of roughly 140-150 mg·l^{-1} for *Saccharomyces* (Agenbach 1977; Bely *et al.* 1991; Reed and Nagodawithana 1991), but technologically even 200 mg N·l^{-1} juice, particularly if sulphur-containing amino acids are lacking, can be inadequate to limit sufficiently the hydrogen sulphide production by yeast (Jiraneck 1999).

Different analytical approaches were used to estimate the amount of yeast assimilable nitrogen. Some methods use the reactions with formol (Fédération Internationale des Producteurs de Jus de Fruits 1965; Becchetti 1988), ninhydrin (Hebbard *et al.* 1993), 2,4,6-trinitrobenzene sulfonic acid (Crowell *et al*. 1985; Ough 1988; Ough and Amerine 1988) or *o*-phthaldialdehyde/N-acetyl-L-cysteine (Dukes and Butzke 1998). Other approaches, not within the reach of all wine laboratories, derive assimilable nitrogen from alpha-amino nitrogen of each amino acid measured by HPLC or Ionic Chromatography and from ammonium.

To meet the requirements of the wine producers in Trentino, in 1997 we set up a spectro-photometric method to measure the promptly assimilable nitrogen (PAN) by yeasts (Corradin 1996; Nicolini *et al.* 2004), adapting the international method of analysis of free amino nitrogen in wort of the American Society of Brewing Chemists (ASBC 1992) for grape juice. It appeared to be a good starting point with regard to health of the analysts, since no formol is needed. For several vintages we investigated, during ripening and at harvest, the content of PAN in grape juices of several varieties from the Trentino region (North-East Italy) and from some other well known grape-growing areas in Italy.

Material and Methods

All grapes were from vineyards trellised and managed according to the usual regional practice, in order to achieve results representative for the Italian vineyard situation.

E x p e r i m e n t A : In 1999, 2000 and 2001, a survey of the content of promptly assimilable nitrogen (PAN) in wine grape juices of the Trentino region (North-East Italy) was carried out. 324 samples from technologically ripe grapes of 14 varieties (Cabernet Sauvignon, Chardonnay, Enantio, Lagrein, Marzemino, Merlot, Müller-Thurgau, Nosiola, Pinot

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gris, Pinot noir, Rebo, Schiava, Syrah, Teroldego) were analysed, and the variability due to the cultivar was investigated. Sampling included the most important varieties and grape-growing areas for quality wines of the region. Juice samples were obtained by manual crushing and mechanical pressing (2.5 bar; Para Press, Paul Arauner GmbH, Kitzingen/Main, Germany) of at least 10 bunches and 2 kg for each grape variety, plot, ripening stage or trellis system investigated. Each sample was filtered through wadding, analysed for total soluble solids (TSS; °Brix), and immediately frozen (-28 °C). The content of PAN was measured a few days later. Grapes have been considered "technologically ripe" when sampled within 5 d prior to the harvest date planned for the individual vineyards by the wineries.

Experiment B: The variability of PAN due to the vintage was investigated in grapes harvested at technological ripeness in 45 vineyards (12 Chardonnay, 5 Cabernet Sauvignon, 1 Lagrein, 7 Merlot, 3 Müller-Thurgau, 3 Marzemino, 2 Nosiola, 6 Pinot gris, 2 Pinot noir, 2 Schiava, 2 Teroldego) in 3 years. Sample preparation was outlined in experiment A.

Experiment C: Effects of grape ripening were studied over 3 years on the basis of 75 ripening curves (Tab. 1), each with 5-7 sampling dates within the 45-50 d before harvest. For sample preparation see experiment A.

Table 1

Distribution per variety and year of vineyards monitored during grape ripening (experiment C)

	1999	2000	2001	2002
Chardonnay	3	7	5	15
Cabernet Sauvignon	1	4	7	12
Lagrein	1	1	1	3
Merlot	4	5	6	15
Müller-Thurgau	2	3	1	6
Marzemino	1	1	3	5
Nosiola	0	2	1	3
Pinot gris	4	0	0	4
Pinot noir	1	1	1	3
Rebo	0	0	1	1
Schiava	1	2	1	4
Syrah	0	0	1	1
Teroldego	0	2	1	3
Total	18	28	29	75

Experiment D: The PAN content in 41 Prosecco juices (1998-1999) from the Venetian region, and in 221 variety juices (2001) of technologically ripe grapes from several Italian regions outside of Trentino was surveyed in industrial wineries. Juice samples were collected just after grape crushing and destemming, focusing on grape varieties and areas which are most typical for quality wines in each region. Juices were analysed for TSS and, immediately thereafter, anti-fermentive (NaN_3, 20 mg·l^{-1}) was added; they were kept at 5-10 °C and were frozen (-28 °C) within 12 h after sampling.

Analyses: The method for the analysis of free amino nitrogen in wort set up by the American Society of Brewing Chemists (A.S.B.C. Technical and Editorial Committees 1992) was applied to grape juices with the following adjustments and specifications (NICOLINI *et al.* 2004), (a) colouring reagent: acetate buffer (4N, pH 5.5), ninhydrin (1 %), fructose (0.3 %); (b) reaction conditions: 100 °C for 20 min with subsequent rapid stabilisation of the temperature at 20 °C for 5-20 min; (c) threonine as reference standard; (d) juice dilution to reach a final concentration of N between 0.5 and 1.5 mg·l^{-1}; (e) absorbance measurement between 10 and 20 min after the final dilution with the hydroalcoholic solution; (f) absorbance values derived graphically by connecting, in perpendicular to the X axis, the peak maximum at 565 nm and the line tangent the 2 minimum values (roughly at 470 and 700 nm) of the plotted spectrum.

Data were statistically analysed with the software package STATISTICA^{TM} for Windows v. 5.1, 1997 (StatSoft Italia S.r.l., Padova, Italy).

Results and Discussion

Results obtained by the modified method (NICOLINI *et al.* 2004) applied to grape juices fitted with values of assimilable nitrogen obtained from the Ionic Chromatography (IC) measurements (NICOLINI *et al.* 2001) of the *alpha*-amino nitrogen of each amino acid and of N as ammonium. The slope of the regression line is close to 1, mainly since the modified method measures ammonium totally, and the intercept with the Y-axis is small and lower than that of the original method (Fig. 1), showing less matrix interferences. Standard deviation (RSD %) of 25 complete analyses, preparation of reagents included, of a juice divided into 25 frozen lots and performed within 5 months was 2.1 %. The values of PAN in juices of different white and red grape varieties (with proline or arginine prevalent, and characterized by PAN contents covering all the range of the assimilable nitrogen which is

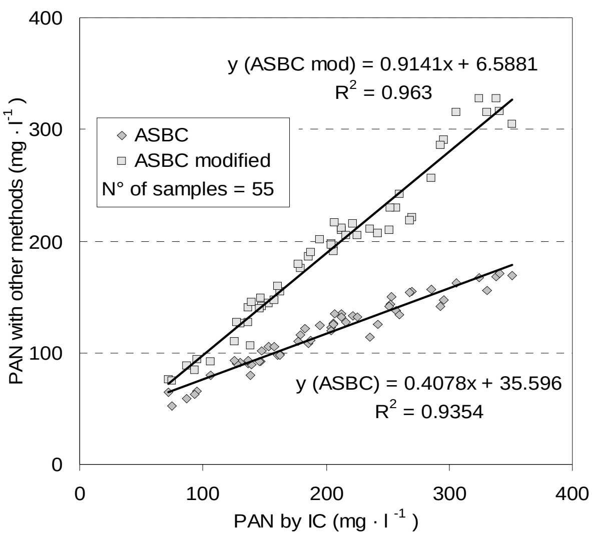

Fig. 1: PAN values of juices measured by the method of the American Society of Brewing Chemists (ASBC) and by our modification (ASBC modified), related to values obtained by Ionic Chromatography (IC) used as reference method.

naturally present in grapes), agree with those derived by IC (Fig. 2). The slopes of the regression lines are similar for all varieties and the values of the intercepts with the Y-axis are always limited, confirming good accuracy and reduced matrix interferences.

E x p e r i m e n t A: The values of PAN and TSS (to characterise grape ripeness) in juices from Trentino are reported in Tab. 2. PAN ranged from 16 to 394 mg·l^{-1}. The large majority of samples was far below the classic deficiency limit of 140 mg·l^{-1} and 25 % of the samples were even below 83 mg·l^{-1}.

Taking into account only varieties with samples in each of the 3 years, the differences among varieties were assessed with the Honest Significant Difference Test of Tukey for samples of unequal number, because of its particular robustness (Tab. 3). Pinot gris juices had the highest mean values of PAN, which were significantly different from all the other variety juices except those from Chardonnay and Pinot noir, both with high PAN. On the contrary, Schiava differed significantly for its low contents of PAN from the just quoted varieties and from Teroldego. These "varietal" differences cannot be attributed exclusively to genetic fac-

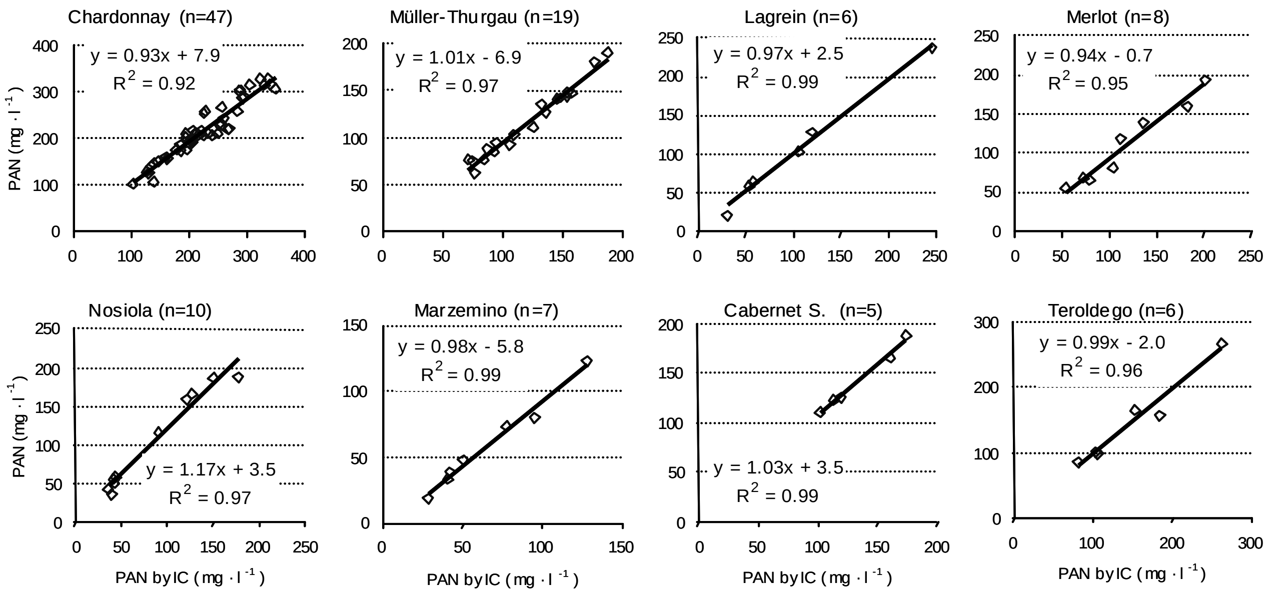

Fig. 2: PAN values of 8 varieties measured by the modified ASBC method related to values obtained by the IC-method. For details see Fig. 1.

T a b l e 2

Values of promptly assimilable nitrogen (PAN) and total soluble solids (TSS) of several varieties harvested at technological ripeness in Trentino in 1999, 2000 and 2001 (* = 2001; ** = 1999 and 2000; W = white; R = red)

Variety	Variety code	Colour of the berry	No. of samples	Promptly assimilate nitrogen (N, mg·l^{-1})					Total soluble solids (°Brix)				
				Median	Min	Max	Lower quartile	Upper quartile	Median	Min	Max	Lower quartile	Upper quartile
Cabernet S.	CS	R	34	115	35	197	88	165	20.9	18.0	23.3	20.0	21.3
Chardonnay	CH	W	54	184	60	324	133	244	19.9	16.5	22.9	18.9	20.6
Enantio**	EN	R	27	134	71	306	100	176	21.3	16.2	23.1	19.2	22.2
Lagrein	LG	R	21	103	21	286	64	159	19.8	17.2	24.4	18.7	21.0
Marzemino	MZ	R	22	99	19	233	56	137	19.1	17.8	21.1	18.6	19.6
Merlot	ME	R	38	85	29	251	64	136	21.8	18.3	24.5	20.7	22.7
Müller-Thurgau	MT	W	18	130	50	200	88	158	17.5	15.1	21.2	16.6	18.4
Nosiola	NO	W	30	103	36	232	60	133	16.5	15.0	19.2	15.5	17.4
Pinot gris	PG	W	24	239	109	356	167	276	19.4	16.6	21.9	18.8	19.9
Pinot noir	PN	R	11	213	124	394	184	237	20.9	17.5	22.6	18.5	21.7
Rebo*	REBO	R	3	112	106	114			21.6	19.0	21.7	19.0	21.7
Schiava	SCH	R	19	89	16	131	55	102	17.3	15.6	19.8	16.5	18.2
Syrah*	SY	R	4	139	28	180	76	167	20.3	16.8	22.8	18.3	21.8
Teroldego	TER	R	19	156	44	285	98	191	21.6	18.6	23.1	20.3	22.1
Total			324	129	16	394	83	185	19.8	15.0	24.5	18.3	21.2

Table 3

Significance of the differences among varieties for the content of PAN (mg · l^{-1}) in juices of technologically ripe grapes from Trentino (p value; Honest Significant Difference Test of Tukey for samples of unequal number; p values > 0.05 not shown)

Variety code	CH	CS	LG	ME	MT	MZ	NO	PG	PN	SCH	TER
No. of samples	(54)	(34)	(21)	(38)	(18)	(22)	(30)	(24)	(11)	(19)	(19)
PAN (mean)	187	116	121	103	125	101	106	230	221	79	145
Chardonnay											
Cabernet Sauvignon	0.000										
Lagrein	0.012										
Merlot	0.000										
Müller-Thurgau											
Marzemino	0.000										
Nosiola	0.000										
Pinot gris		0.000	0.000	0.000	0.000	0.000	0.000				
Pinot noir		0.001	0.003	0.000	0.005	0.000	0.000				
Schiava	0.000							0.000	0.000		
Teroldego								0.000		0.021	

tors, since, in the present survey, each sample results from interactions with its "terroir". However, these differences have a clear significance for the winemaking of different varieties, because of the large representativity of the samples with respect to the real production of the region. *E.g.*, the low PAN content in the autochthonous cvs. Schiava and Marzemino accounts for the frequent overproduction of sulphur compounds in wine causing unpleasant flavours.

Experiment B: The variability due to the vintage was assessed by determining the composition of variety juices from 45 vineyards for 3 years. Significant differences among years (LSD test) were observed only for the variables TSS and PAN (Tab. 4). In 1999, TSS were lower compared to 2000 and 2001, but there was no difference for PAN. In 2000, PAN was significantly higher than in 2001. The differences among the average values per year and the values of the standard deviations seem to be larger for PAN than for the other variables normally used for quality control of grape at harvest. This confirms our previous findings both for Chardonnay grapes grown on 7 different trellis systems (pergola, spur cordon, lyra, GDC, vertical cordon free, spur cordon with mechanical pruning, simple curtain) in the same vineyard (Tab. 5) and in numerous samples from a Müller-Thurgau vineyard (NICOLINI *et al.* 2004). In the light of the above-cited role PAN plays for the fermentation aroma, these observations contribute to explain why grapes of a variety with "neutral" aroma, with the same "basic" composition, and processed with the same winemaking procedures frequently give wines with significantly different aroma.

Experiment C: Due to the large variability of the PAN values in different years, varieties, and plots, data were expressed as percentage of the final content of PAN at harvest for each mono-variety vineyard. Fig. 3 shows a significant, negative and linear correlation indicating an overall decreasing trend of PAN before harvest. This trend is the result of the decrease of ammonium and the increase of the amino acids included in PAN (LAFON-LAFOURCADE and GUIMBERTEAU 1962; KLIEWER 1968, 1970; MILLERY *et al.* 1986; VERSINI *et al*. 1995; NICOLINI *et al.* 2004). The R^2 value of the regression line is definitely low, and does not increase with functions of higher order. So, the trend of PAN during ripening does not seem to be so easily generalizable as for TSS and total acidity. This could be due to the kind of survey we carried out using a very large number of varieties, vineyards, different kinds of canopy management and agronomic practices. Morover, it might be affected by the above-mentioned larger variability of PAN in comparison with that of other classic analytical parameters. This variability urges to reconsider the procedures of sampling in vineyard if PAN is the target.

Experiment D: The results of the survey of the PAN content in juices from several Italian regions are reported in Tab. 6. Discussion will focus on varieties with a higher number of samples in relation to their main growing area. As previously outlined, "variety" in this context comprises the interaction variety- terroir.

Among the varieties from Central and South Italy, Negramaro, Malvasia nera and Montepulciano showed sat-

T a b l e 4

Effect of vintage on the composition of single-variety juices from Trentino
(Values with the same letter do not differ significantly, $p < 0.05$. Capitals were used when $p < 0.01$)

Year	No.	pH		Total acidity ($g \cdot l^{-1}$)		TSS (°Brix)	
	obs.	Mean	SD	Mean	SD	Mean	SD
1999	45	3.22	0.15	8.19	1.45	19.13 b	1.74
2000	45	3.28	0.21	7.88	1.61	20.30 .A	1.75
2001	45	3.26	0.18	7.61	1.84	19.93 a	1.83
Year	**No.**	**Tartaric acid ($g \cdot l^{-1}$)**		**Malic acid ($g \cdot l^{-1}$)**		**PAN ($mg \cdot l^{-1}$)**	
	obs.	Mean	SD	Mean	SD	Mean	SD
1999	45	6.43	0.79	4.22	1.29	161 ab	71
2000	45	6.34	0.87	4.00	1.21	173 A	79
2001	45	6.37	0.98	3.79	1.47	134 b	59

T a b l e 5

Composition of musts of Chardonnay grapes grown in a vineyard on 7 different trellis systems, each with 2 replicates

Variable	Mean (n=7)	Max	Min
PAN ($mg \cdot l^{-1}$)	257	341	198
TSS (°Brix)	19.4	20.0	18.9
Total acidity ($g \cdot l^{-1}$)	10.0	10.4	9.0
pH	3.25	3.30	3.22

isfactory contents of PAN; however, one forth of the samples of Montepulciano were below 67 $mg \cdot l^{-1}$. In the region of Marche, all samples of Verdicchio were very close to their not particularly high median value. As for Northern Italy, among the varieties from Piedmont, Barbera showed the highest content of PAN, while Nebbiolo had definitely low amounts irrespectively of ripeness (ranging from 16.5 to 25 °Brix). In Lombardy, Syrah had high levels of PAN, and Barbera confirmed its trend to fairly high PAN contents, with only 1 out of 5 samples below 120 $mg \cdot l^{-1}$. In Franciacorta, the PAN content of Chardonnay agreed with that measured by Fregoni *et al.* (2003) in 36 juices for sparkling wine in 2001 and 2002. Among the varieties Lambrusco, in Emilia Romagna, Sorbara had higher contents than Grasparossa and Salamino. In Veneto, 75 % of the Prosecco juices, all for sparkling wine, were below 144 $mg \cdot l^{-1}$ of PAN, with a mean value of 112 $mg \cdot l^{-1}$. The Prosecco juices seem to be characterized, in general, by lower assimilable nitrogen than those of Chardonnay for sparkling wine from neighbouring Trentino. In fact, the mean contents of Chardonnay juices for sparkling wine in Trentino are rarely below 200 $mg \cdot l^{-1}$ (*e.g.* 1986, n = 30, 207 $mg \cdot l^{-1}$; 1987, n = 30, 227 $mg \cdot l^{-1}$; 1988, n = 30, 215 $mg \cdot l^{-1}$; 1991, n = 14, 191 $mg \cdot l^{-1}$; 1995, n = 19, 246 $mg \cdot l^{-1}$; 1996, n = 20, 273 $mg \cdot l^{-1}$). Ribolla, the typical white variety from Collio in Friuli, had fairly high contents of assimilable nitrogen.

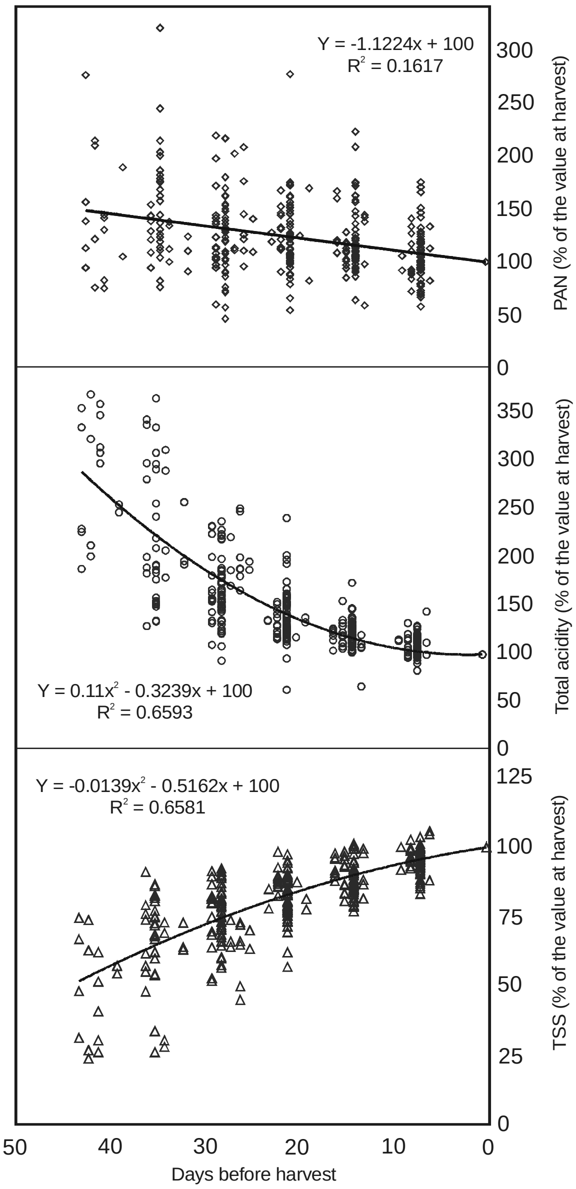

Fig. 3: PAN values, total acidity and TSS of 424 grape samples before harvest.

Table 6

PAN and TSS in juices of grapes harvested at technological ripeness in several Italian regions in 2001 (n.a. = data not available)

Region / area	Variety	No. of samples	PAN ($mg \cdot l^{-1}$) Lower quartile	Median	Upper quartile	TSS (°Brix) Lower quartile	Median	Upper quartile
Abruzzo	Montepulciano	17	67	152	195	21.4	21.9	23.1
Alto Adige	Chardonnay	1		209			21.7	
	Cabernet S.	1		95			22.9	
	Merlot	1		128			21.5	
	Pinot blanc	5	192	196	198	21.5	21.7	21.7
	Pinot noir	2	126	210	294	23.2	24.0	24.9
	Traminer	5	179	231	250	22.6	22.6	23.7
Basilicata	Aglianico	1		113			25.5	
	Tamurro	2	52	59	65	23.4	24.2	25.0
Emilia Romagna	Albana	10	81	104	140	20.7	21.7	23.5
	Ancelotta	1		119			19.6	
	Barbera	1		127			23.9	
	Cabernet franc	1		101			18.9	
	Chardonnay	1		138			24.5	
	Croatina	1		107			23.9	
	Grasparossa	7	100	144	159	17.8	19.4	20.6
	Salamino	8	112	126	159	16.1	17.4	18.3
	Sorbara	7	170	186	220	17.3	17.7	19.0
Lombardia / Franciacorta	Barbera	3	121	173	180	22.1	24.0	24.1
	Chardonnay	6	100	253	263	18.0	18.9	19.6
	Cabernet S.	4	182	187	200	20.9	21.4	22.2
	Fiano	1		72			18.1	
	Manzoni bianco	1		160			22.6	
	Marzemino	2	54	67	79	21.8	21.8	21.9
	Nebbiolo	1		94			20.9	
	Pinot blanc	8	114	179	217	16.3	16.9	20.0
	Pinot noir	4	108	114	155	20.4	20.8	21.6
	Rebo	1		129			20.4	
	Roussanne	1		112			17.1	
	Sangiovese	1		87			22.2	
	Syrah	5	207	242	273	20.8	22.0	22.7
	Viognier	2	67	122	176	15.6	17.1	18.5
Lombardia / Oltrepò	Barbera	2	34	116	197	19.0	20.4	21.8
	Croatina	2	51	82	113	23.4	23.8	24.1
	Pinot noir	2	118	239	360	16.8	18.0	19.2
Friuli	Chardonnay	1		208			19.9	
	Cabernet S.	8	63	88	189	19.2	21.5	22.8
	Merlot	6	59	86	102	19.8	20.3	23.0
	Refosco	3	83	137	200	18.7	19.5	20.7
	Ribolla	8	129	136	177	18.1	18.8	19.2
	Sauvignon b.	3	170	181	284	19.8	19.9	21.4
	Tocai	3	83	127	140	17.2	18.0	20.4
Marche	Verdiccio	5	96	100	105	22.0	22.1	22.9
Piemonte	Barbera	12	119	160	207	20.5	21.8	22.6
	Cortese	9	86	98	119	17.4	18.4	18.7
	Dolcetto	6	42	68	107	19.1	19.7	21.2
	Freisa	1		91			21.3	
	Grignolino	4	47	65	97	21.4	21.9	22.1
	Nebbiolo	7	13	62	80	17.5	22.5	25.1
Puglia	Malvasia nera	5	129	133	141	24.1	24.1	25.1
	Negramaro	6	137	181	192	20.7	21.2	21.6
	Primitivo	4	93	148	205	22.7	26.2	28.0
Sardegna	Cannonau	4	69	108	163	23.9	25.1	26.8
Sicilia	Nero d'Avola	2	180	213	245	20.1	20.6	21.2
Veneto (1998-1999)	Prosecco	41	75	117	144	n.a.	n.a.	n.a.

Conclusion

It is known that viticultural management can affect the content of ammonium and amino acids in grape juices (SPONHOLZ 1991). In the present work we studied some agronomic factors with regard to winemaking, using promptly assimilable nitrogen by yeasts as a fundamental parameter. In addition, we surveyed the yeast assimilable nitrogen status of the juices of several Italian grape-growing areas and varieties.

Roughly 600 juices from technologically ripe grapes from several Italian regions were analysed. The natural contents of assimilable nitrogen averaged out at 136 $mg \cdot l^{-1}$, ranging from few tens of $mg \cdot l^{-1}$ up to almost 400 $mg \cdot l^{-1}$. The mean values were definitely lower than those (213 $mg \cdot l^{-1}$) reported by BUTZKE (1998) for roughly 1500 musts from California, Oregon and Washington in 1996. 58.3 % of the Italian samples were below the classic deficiency threshold of 140 $mg \cdot l^{-1}$, in comparison with 13 % of the quoted American juices. Significant differences among varieties, even if with high variation among individual juices, and among different vintages were observed. It is interesting to note that Chardonnay and Pinot noir are among the richest in assimilable nitrogen both in the United States and in Italy.

With the proposed method, roughly 40 samples per day can be analysed, so that, in the light of the slight decrease observed with ripening, the PAN content in grapes of many vineyards can be checked few days before harvest. This provides information not only for fermentation practices but also about the nutritional status of vineyards (VAN LEEUWEN *et al*. 2000; FREGONI *et al*. 2003). However, sampling has to be done carefully, since the variability observed in vineyards for PAN seems to be larger than those for sugars and total acidity.

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