FOOD FLAVORS: GENERATION ANALYSIS & PROCESS INFLUENCE

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37B

(11,12). The contribution from the Ehrlich mechanism is much lower than that from carbohydrate degradation (13).

The higher alcohols are present in concentrations above their organoleptic perception threshold. At concentrations below 300 mg/l, they certainly contribute to the desirable complexity of wine. When their concentrations exceed 400 mg/l, the fusel alcohols are regarded as a negative quality factor. Lee et al. (19) found a higher content of the total fusel alcohols in red wine (256 mg/l in Leon Millot wine) than in white-wine (149 mg/l in Riesling); being, in any case higher in hybrids (e.g. 507 mg/l in Concord wine).

The **fatty-acids** are formed earlier during the alcoholic fermentation and in higher concentration than their corresponding fatty acid ethyl esters. Relatively few organic acids in wine are volatile enough to contribute to its odor. Odorous acids are acetic acid (vinegary), butanoic acid (spoiled butter) lactic acid and caprylic acid (goaty). Goaty flavour is typical for caprylic acid as found in beer (85). With the exception of acetic acid, their amounts in wines are usually below perception threshold. Medium contents found in a Chardonnay wine for octanoic acid are 7.9 mg/l; std. dev. 4.4 mg/l and for hexanoic acid 5.8 mg/l; std. dev. 2.9 mg/l (84). The contents of all fatty acids (C-4 to C-10) increase during fermentation, whereas those of C-16 to C-18-acids decrease (20).

Ethyl esters of straight-chain fatty acids and **acetates** of higher alcohols are the dominanting esters in wine, and they are formed during the alcoholic fermentation (Fig. 2). The yeasts synthesize the acetates in the same way as the fatty acids; water hydrolysis of the acetyl-CoA-derivative gives the fatty acids, and similar, ethanol hydrolysis gives the acetates.

Volatile organic **sulphur compounds** produced by alcoholic fermentation are of outstanding importance for aromas because, for the most part, they have extremely low perception thresholds. The most important S-containing compound in wine is hydrogen sulphide, which has a recognition threshold below 1 μg/l. It appears predominantly in high amounts during the fermentation of grapes with free elemental sulphur (21) and causes the "rotten egg" odor. Other odor-intensive sulphur compounds in wine are thioethers (dimethylsulphide, diethylsulphide), thiols (ethanethiol, 4-methylthio-1-butanol, 3-methylthio-1-propanol), thiolanes (2-methylthiolane-3-one, 2-methylthiolane-3-ols), and esters of sulphur-containing acids (methyl and ethyl-3-methylthiopropionate) (14,5,15,86). The odor of trans-2-methylthylthiolane-3-ol is onionlike, that of ethanethiol is fecal.

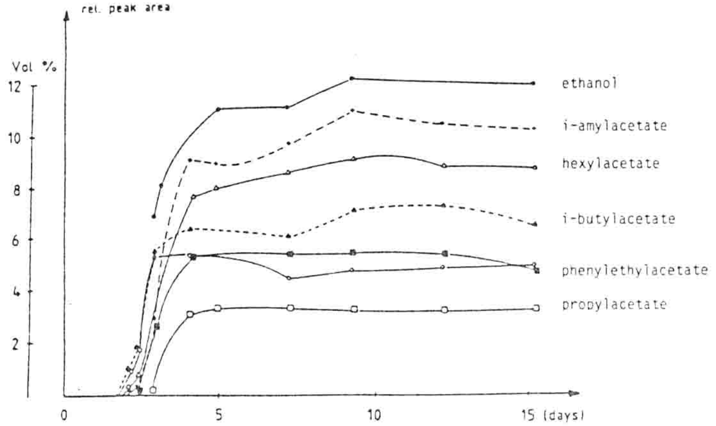

Fig. 2: Formation of acetates ("fermentation compounds") during fermentation (Rapp et al.)

The most volatile **phenols** which are found in wines are not present in grapes, except acetovanillone (5). They result either from yeast or bacterial metabolism, of corresponding benzoic acid and cinnamic acid or from hydrolysis of higher phenols and phenol glycosides (87). For example, the volatile phenols 4-vinyl-guajacol and 4-vinyl-phenol can originate from the cinnamic acids p-cumaric acid and ferulic acid by enzymic or thermal decarboxylation (1). These phenols can influence wine odor. Their content in Moscato rosa-wines ranged from 2 to 8 µg/l, in wine of white varieties (e.g. Traminer) levels were found of about 1 mg/l (17,88,89).

The process of volatile **nitrogen compounds** are also determined by wine production. Among the most aboundent substances, N-(2-phenylethyl)-acetamide ranges in Moscato Rosa-wines between 1 to 23 mg/l while N-(3-methylbutyl)-acetamide ranges between 15 to 72 mg/l.

Many remarkable **lactones** from wines thus far known seem to arise during fermentation through reactions involving 4-oxobutyric acid and, to a lesser extent, 2-oxo-glutaric acid (18). Among the many volatile constituents of wine, the lactones, particularly gamma lactones, occupy a place of prominence not only in terms of their contribution to the total vinous aroma and bouquet, but also because of their physiological properties. Other δ- and γ-alkyllactones, the so called "peach lactones", can be present in wines but they could be considered as worthless contributors to the total aroma as shown by Etievant et al. (90).

Terpene compounds, as a group, form an important part of the grape bouquet; they belong to the secondary plant constituents, of which the biosynthesis begins with acetyl-CoA. Microorganisms are also able to synthesize terpene compounds (16), but the formation of terpenes by Saccharomyces cerevisiae has not yet been observed. These compounds are not changed by yeast metabolism during fermentation (Fig. 3) (2,3,8) except for geraniol (91) and nerol (92). The monoterpene compounds are, therefore, suitable for the varietal characterization of wines made from different grape varieties (1,3).

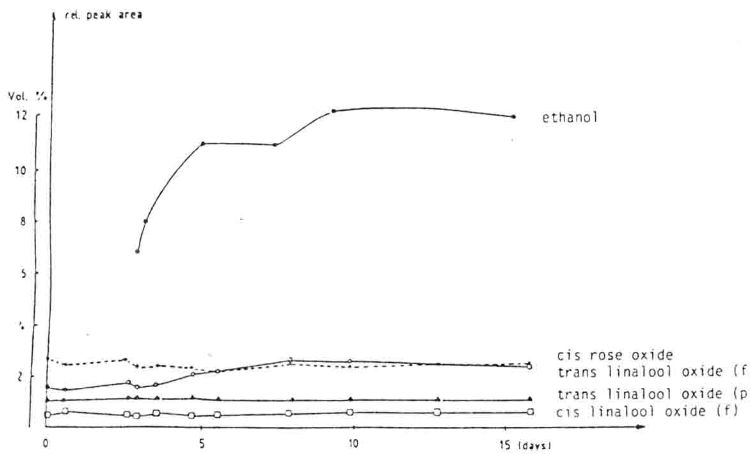

Fig. 3: Behaviour of monoterpene compounds ("grape aroma compounds") during fermentation (Rapp et al.)

INFLUENCE OF VARIOUS FACTORS ON THE PRODUCTION OF FERMENTATION COMPOUNDS

It is fairly well accepted that several factors can change the "fermentation bouquet" of wines. The type of yeast has a great influence on the production of fermentation compounds, but even more important are the clarification and fermentation conditions, including the treatments and the composition of the musts (pH, nitrogen compounds, nitrogen contents).

Yeast strains selected for alcoholic fermentation of grape juice have been found to cause appreciable variation in the quality of the wines and in their contents of volatile substances (22). In fact some compounds (e.g. hydrogen sulfide, aldehydes and acetic acid) were present at levels sufficiently high to influence the quality of some wines negatively, whereas in most wines of higher quality, the fatty acid esters were present in concentrations and ratios which are typical of better quality wines. Saccharomyces cerevisiae strains, in general, produce higher quality wines than those of Saccharomyces uvarum, Saccharomyces bayanus and Saccharomyces chevalieri. The total higher alcohol production of 24 selected strains of these subspecies did not vary considerably (22). The same is true for individual higher alcohol contents, except for the 2-phenylethanol levels produced by three Saccharomyces uvarum strains. The highly significant negative correlation coefficient between wine quality and 2-phenylethanol content may suggest that, at the possible extraordinary high levels as found, this alcohol could make a negative contribution to the quality of the wines (22). Also Cavazza et al. (23) found no significant differences in the levels produced of higher alcohols except for 2-phenylethanol and acetamides by different yeast strains. Two of the three 2-phenylethanol high producing strains were classified as Saccharomyces uvarum, the other one as Saccharomyces cerevisiae.

The production of higher alcohols depends on the degree of juice turbidity and fermentation temperature. In clear filtered juice, however, the production of higher alcohols is independent of fermentation temperature (24). The results of numerous experiments with filtered juice and strictly anaerobic fermentation indicated that the concentrations of the 3 main higher alcohols (2-methyl-propanol-1, 3-methyl-butanol-1, 2-phenylethanol) amounted to a rather constant total of 90 mg/l at 13 °C and 15 °C, as well as at 25 °C. On the other hand, in wine from settled juice, fermented at 13 °C and 25 °C, the total higher alcohol concentration amounted to 170 and 375 mg/l, respectively.

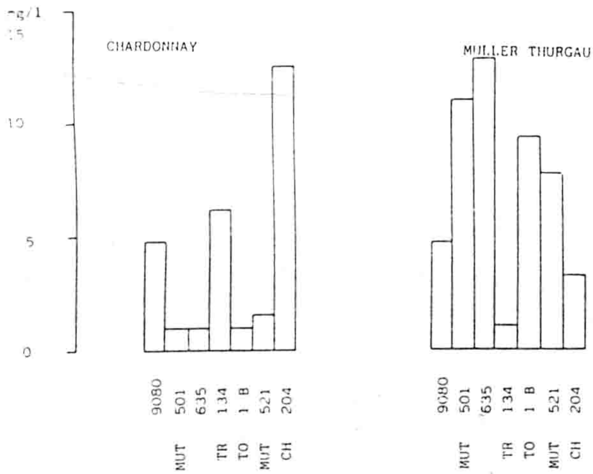

Fig. 4: Formation of isoamylacetate during fermentation at different musts and yeasts (Cavazza et al.; 23)

The major factors affecting ester content of wine are medium composition (Fig. 4) and fermentation procedure. Variations in media include carbon source, nitrogen supply, and pH. Important fermentation procedure factors include fermentation temperature, carbon dioxide concentration, oxygenation of the media, and yeast strain selection.

Differences in ester production due to yeast species or strains have been reported (Table 1) (22,23,28,29). It has been shown in several studies (25,22) that the contents of individual fatty acid esters such as isoamyl acetate, ethylhexanoate, ethyloctanoate are normally higher in wines with higher quality ratings. The positive contributions of some esters to wine bouquet have, in fact, been demonstrated conclusively (25,22).

The fruity esters (isoamyl acetate, isobutyl acetate, ethyl butyrate, hexyl acetate) are produced and retained at the lower fermentation temperature (10 °C). The higher-boiling, more aromatic or "heady" esters (ethyl octanoate, 2-phenylethyl acetate) are produced and retained in the wine in greater amounts at higher fermentation temperatures

Table 1: Influence of yeast strains on ester content of wine (Zeemann et al.; 22)

yeast species and strain	ethyl-acetate	i-amyl acetate	2-phenyl-ethylacetate	ethyl-hexanoate	ethyl-octanoate
S. cerevisiae 1	56	12	0.22	1.7	1.3
S. cerevisiae 14	60	7	0.10	1.1	1.1
S. cerevisiae 372	56	7	0.21	1.3	1.1
S. cerevisiae 373	62	14	0.38	2.7	1.5
S. bayanus 390	36	10	0.36	1.4	0.9
S. uvarum 355	67	13	2.2	3.2	1.1
S. uvarum 402	51	4	4.6	0.5	1.0
S. uvarum 400	53	1	2.1	0.2	0.2

(15 to 20 °C) (26). The aroma of a wine produced by low fermentation temperatures is distinctively characterized by a fruity and soap like odor typical for some esters (27).

Levels of fatty acids and their ethyl esters depend on the skin contact time (30), in some cases they could be higher in wines from pressing fractions so as it is usually for the acetates (93). During the second fermentation (sparkling wine production), the decrease of the fatty acid esters depends on the yeast level (31).

Cavazza et al. (23) could separate some yeast strains with the aid of discriminant analysis based on the concentrations of esters and acids (7 variables). Nevertheless, Marchetti et al. (32), investigating 28 wines of different varieties, showed that the influence of the must was greater then any of 16 different yeast strains on the production of fermentation aroma compounds (Fig. 5).

AMINO ACIDS OF MUST

The characterization of amino acid composition in grape musts and wines is of great interest because such compounds represent an important source of directly assimilable nitrogen and are also precursors in the synthesis of some volatile compounds (e.g. fusel alcohols) in alcoholic fermentation.

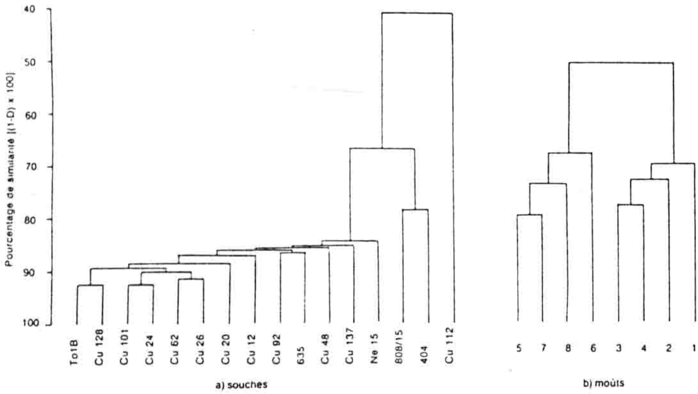

Fig. 5: Cluster analysis by means of higher alcohols
(8 different musts; 16 different yeast strains; Marchetti et al.; 32)

In the 1930s, amino acids were found in musts for the first time. Since then, many authors have dealt with the amino acid composition in grape berries and musts (33,34, 35,36,37,38,39,40,41,42). According to Kliewer (35) 60 to 90% of nitrogen content in grape juices is present in form of amino acids. Grape varieties differ from each other merely for the amount of certain common amino acids. Cantagrel et al. (50) compared the amino acid contents of several varieties and found higher levels in Pinot and Syrah. On the other hand, the socalled "Mediterranean" varieties (Carignan, Cinsault, Grenache) showed a lower content, particularly Grenache.

The most abundant amino acids are generally arginine, glutamine, proline, alanine, glutamic acid, threonine, serine and γ-amino-butyric acid. They represent 80 to 90% of the total amino acid content, and in certain varieties, proline predominates up to 80 to 90% of that level (43). The variations of amino acid profiles in musts depend upon variety, crop level, density of plantation, fertilization and composition of soil, grape ripening and degree of Botrytis cinerea infection, as well as upon harvesting procedure and climatic characteristics.

The amino acid content of grape berries increases with the advancement of **ripening**, with a very different extent (Table 2) (33,35,39,44,45). The decrease in **yield/plant** brought about an increase in total nitrogen content and in free amino acids level, particularly of that of proline (34). An important increase in amino acid concentration, was observed by increasing the density of plantation (1989). Kliewer (34) proved that the ratio of arginine/proline content in grape juice increases with the crop level at ripeness.

Table 2: Amino acids in grapes during ripening
(Riesling 1976; mg/l; Rapp; 57)

amino acid	9.8.76	23.8.76	8.9.76	22.9.76
Asp	48	60	75	74
Thre	23	50	109	128
Ser	35	70	126	155
Glu	69	106	140	160
Glu-NH_2	240	630	1000	1030
Pro	8	46	165	278
Ala	10	60	100	145
Val	9	7	18	57
Met	3	5	11	20
iLeu	8	13	18	71
Pheal	14	17	21	73
Lys	3	5	5	10
Arg	150	255	490	660
total amino acid	778	1490	2540	3320

Nitrogen fertilization influences the amino acids in must (68): Significant differences were found mainly between zero and the 112 kg/ha treatment (Table 3). Nitrogen fertilization above this level did not seem to affect the nitrogen composition of the fruit much, even though nitrate petiole values increased consistently with the amount of nitrogen applied to soil.

Botrytis cinerea infection of grapes deeply influences the amino acid content of berries and/or juice (Table 4). The decrease of total amino acid amount can reach up to 80%, though it could differ for each amino acid (39, 40). Such variation of amino acid profile can influence the production of some fermentation components.

Table 3: Effects of nitrogen fertilization of grapevines on amino acid in grape juice (Ough et al.; 68)

amino acid	nitrogen fertilization (kg/ha)		
	0	112	448
Ala	61	165	159
Val	15	32	28
Leu	25	47	51
iLeu	13	23	28
Ser	26	64	63
Threo	19	68	50
Asp	42	84	70
Glu	219	437	400
Pro	311	627	542
Arg	314	1068	1083
Total N	329	701	737

The amino acid content increases by increasing **pressure** in the pressing of grapes (46) and, to a smaller extent, by heating crushed grapes (47). **Mechanical harvesting** brings about an increase of amino acids in musts if compared with manual harvesting, as found by Cantagrel et al. (50): For Grenache the increase ranges between 60 to 70%, for Carignan between 60 to 80%. In that respect, all amino acids show the same tendency.

By comparing the influence of **climate** in the same area (Beaujolais) throughout two vintage years (1977 and 1978) on a specific variety (Gamay), Cantagrel et al. (50) found lower amino acid levels in musts of 1978 than in those of 1977. According to Flanzy et al. (48) and Schrader et al. (49), higher levels of amino acids can be found in musts of cooler years than in those of warmer and sunny years. Apparently in cooler years a smaller amount of proteins is synthesized in the not-sufficiently ripening berries.

Table 4: Effects of Botrytis cinera on amino acids (mg/l) in grape juice Rapp et al.; 39)

amino acid	Castor control	Castor attacked by Botr. cinerea	Bacchus control	Bacchus attacked by Botr. cinerea
His	23	14	59	-
Lys	-	10	9	66
Arg	870	78	1100	391
Asp	53	24	24	32
Threo	126	10	80	30
Ser	357	35	248	154
Glu	112	74	47	179
Pro	46	18	82	-
Ala	658	45	125	116
Val	23	5	19	10
iLeu	30	3	42	12
Pheala	30	10	72	25
total amino acid	2461	348	2080	1089

Seeber, Versini et al. (51) investigated the variation of amino acid composition in Chardonnay musts of different **vintage years** (1986, 1987, 1988) from 31 growing areas in a relatively small geographical region (Trentino, Italy) (Table 5). The musts were all obtained exactly with the same technology. By means of cluster-analysis, certain amino acids were grouped after similar content variation, notwithstanding a different biological origin, in some cases: threonine/serine; isoleucine/leucine/valine; methionine/phenylalanine; proline/γ-amino butyric acid. Good linear correlations were found in musts between the content of threonine related to that of serine, as well as to that of tyrosine; arginine related to asparagine and to histidine, proline related to γ-amino butyric acid and to the sum of amino acids. No correlations were found between sugar contents or crop load/stock (under limited variations) and the sum of amino acids (63).

With the aid of a proper stepwise feature selection procedure a pretreatment of data devoted to identifying the variables with highest discriminant ability, six variables were selected, according to the following order: glutamic acid, aspartic acid, proline, leucine, alanine und serine. The discriminant analyses based only on the content of the

Table 5: Amino acids of Chardonnay musts and volatile compounds of the wines (73 samples of 31 regions; 3 vintages: Seeber et al.; 51)

amino acid	1986 mean (mg/l)	1987 mean (mg/l)	1988 mean (mg/l)
aspartic acid	67.9	47.6	44.8
threonine	73.4	73.2	77.8
serine	114.6	103.5	106.3
asparagine	24.7	29.3	26.4
glutamic acid	91.2	135.4	130.9
glutamine	323.7	288.8	227.6
proline	693.1	823.0	614.3
valine	24.7	24.6	29.1
iso-leucine	12.3	11.5	16.9
phenylalanine	25.2	21.7	29.8
arginine	243.5	249.6	278.8

compound	1986 mean (mg/l)	1987 mean (mg/l)	1988 mean (mg/l)
1-propanol	26.0	19.7	19.4
2-methyl-1-propanol	41.3	40.3	46.7
2-methyl-1-butanol	24.9	20.9	28.7
3-methyl-1-butanol	124.9	110.0	137.6
1-hexanol	1.3	1.8	2.0
benzyl alcohol	0.02	0.04	0.03
2-phenylethanol	15.5	18.5	20.3

six amino acids showed a significant differentation between the vintages 1986, 1987, and 1988, but no differentiation among the growing regions was possible (Fig. 6). No significant overlap between 1986 and 1988 vintages was evident. While 1987 exhibited a slightly lower correct predication rate, its characteristics were intermediate between those of 1986 and those of 1988 (51). No discriminations among vintage years were found on the basis of amino acid content in the wines, these values being widely

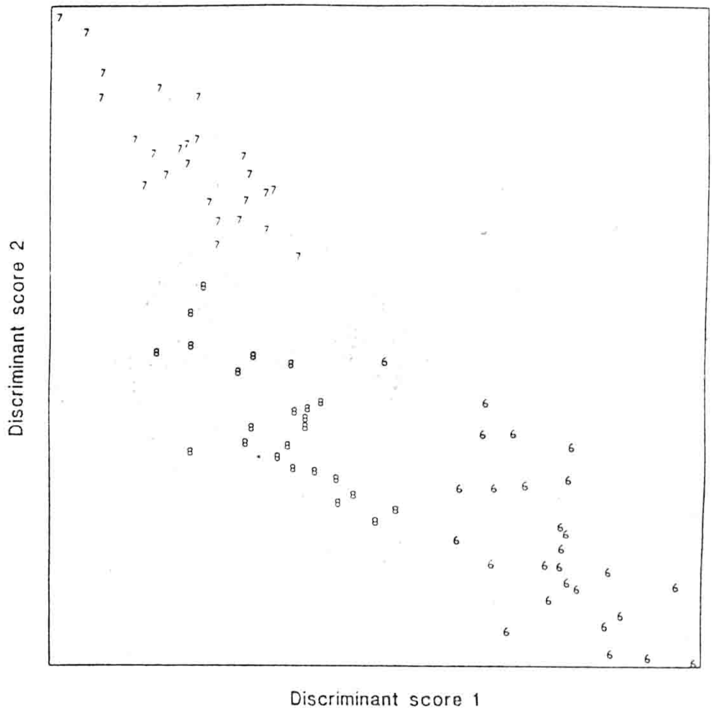

Fig. 6: Discriminant Analysis of 73 Chardonnay wines of 31 regions (amino acids) (Seeber, Versini et al.; 51)

dependent upon yeast metabolism. On the other hand, such discrimination was possible by using other variables (e.g. certain metals and volatiles)

VARIATION OF AMINO ACID LEVEL IN RELATION TO THE ALCOHOLIC FERMENTATION AND AMINO ACID COMPOSITION OF WINES.

Many facts influence the amino acid levels in wine: climate, soil, variety and wine-making technology. The variety seems to have the greatest influence (50). Among the 75 variables considered by Symonds and Cantagrel (65), proline is one of the most significant parameter together with shikimic acid, some esters, and magnesium for the statistical discrimination of wines from five varieties (Syrah, Gamay, Pinot noir, Malbec, Carignan). 42 wines of 8 different varieties from Portugal were separated by Vasconcelos et al. (66), with similar statistical procedures, using only the amino acids as variables. Also Etievant et al. (67) considered some amino acid levels as useful variables for the varietal and geographical classifications of some French red wines.

Peynaud and Lafon-Lafourcade (62) explained the amino acid metabolism through:
- direct assimilation
- deamination, decarboxylation, and production of ammonia, the preferential nitrogen source for yeasts
- transamination.

During the alcoholic fermentation, a large variation of nitrogen content in must takes place. The growing yeasts assimilate a part of many N-compounds, so that the decrease of soluble nitrogen can range from 30 to 80%. Some of the free amino acids can decrease even to a larger extent (75 to 90%). At the beginning of fermentation, the contents of many amino acids are strongly reduced, whereas at the end of the process some increase considerably because of being desorbed from yeasts or because of autolysis of the cells (41, 43, 44, 50, 52, 53, 54, 55, 56, 57, 58, 59). Cantagrel et al. (50) assessed the amino acid content of 70 musts and of the corresponding wines. In two cases, the amino acid content in the wines was higher than that in the musts, whereas in seven wines there was a decrease varying between 0 to 30% and in 29 wines even higher than 60%. By fermenting the same musts with the same yeasts in different wineries, a variable decrease of amino acid content was observed. This was explained (69) by the influence of different winemaking processes (e.g. fermentation temperature, and speed).

Not all amino acids concentrations decrease during fermentation, most wines have a higher content of lysine, glycine and cysteine than the corresponding musts (41,50,52, 57,58). The proline level in must may either increase or decrease - sometimes considerably - or keep constant because of the fermentation metabolism of Saccharomyces cerevisiae, as Rapp et al. (55,57,58) and other researchers (36,44,50,51,60) found, and that is in relation to the total free amino acid content, less or higher than approximately 700 to 1000 mg/l (Table 6).

The fermentative variation of proline seems to be considerably influenced also by the variety and the climatic characteristics of the vintage year (50): Syrah musts had in

1978 similar average level of this amino acid in Rhône Valley so as in Tarn Valley (273 mg/l and 292 mg/l), but proline respective contents in corresponding wines were very different: 433 mg/l for the first area and 1944 mg/l for the second one. Analogous behavior tendencies were observed in Beaujolais region, if taking into account the subsequent vintage years 1977 and 1988, with respective mean values in the musts of 196 mg/l and 141 mg/l and of 205 mg/l and 436 mg/l in the corresponding wines.

Table 6: Changes in content of amino acids during fermentation (Rapp, 57)

	Riesling		Silvaner	
amino acid	must	wine	must	wine
His	34	2	59	11
Lys	2	13	27	60
Arg	605	76	1130	707
Asp	75	9	94	13
Thr	80	3	136	26
Ser	188	11	392	132
Glu	106	8	77	47
Pro	122	53	115	308
Ala	56	17	155	25
Glyc	+	8	8	38
Val	24	3	76	26
Met	13	8	8	10
iLeu	46	5	70	16
Leu	55	19	56	15
Tyr	9	12	76	56
Pheal	61	10	112	36
Total amino acid	1340	640	2645	1730

In the experimental fermentations with different musts we found (55,57,58) that each amino acid is metabolized by yeasts with a different intensity: glutamine, asparagine, serine, glutamic acid, aspartic acid and arginine are the favorite sources for yeast growth compared to the others such as cysteine, methionine, phenylalanine, glycine, and tryptophane. Similar results were published by Amerine and Joslyn (61). The yeast metabolism of nitrogen sources is also dependent upon the pressure in the fermentation vessel; less ammonia, leucine, and histidine are consumed in such a situation (64).

INFLUENCE OF AMINO ACID AMOUNT IN MUSTS ON THE FERMENTATIVE PRODUCTION OF VOLATILE AROMA COMPOUNDS IN WINES

Higher alcohols

As demonstrated in many research works (33,50,51,52,55,57,58,59,70), the fusel alcohol concentration in wine can be determined by the nitrogen content in must, so explaining the importance of amino acid composition in this connection.

1-propanol

Seeber, Versini et al. (51) found (Fig. 7) in Chardonnay musts and corresponding wines of three consecutive vintage years, a general positive linear correlation (significance > 99%) between 1-propanol concentration, in the wine and the total free amino acid content in must, the latter varying from ca. 1000 mg/l to ca. 3000 mg/l. Similar results were also found by Ough et al. (68). They showed that nitrogen fertilization of wines has a determining effect on higher alcohol level and, among other things, increases 1-propanol concentration. Also, addition of $(NH_4)_2HPO_4$ to the must increased the content of 1-propanol, as shown by Äräpäa et al. (70) and confirmed by Margheri, Versini et al. (71). By comparing 70 musts and the corresponding wines, Cantagrel et al. (50) found a non linear correlation between threonine concentration in must and 1-propanol level in wine, although it significantly increased up to amino acid concentrations of approximately 150 mg/l. The lower quantity of 1-propanol in wines of Grenache, Cinsault and Carignan - the above-mentioned "Mediterranean" varieties - could be explained by the likewise lower levels of amino acid nitrogen in their musts. This fact is confirmed by the results for other varieties, e.g. Gamay and Pinot noir, which show opposite composite situations (56).

The different volatile compositions, particularly of 1-propanol in wine obtained from mechanically harvested grapes in comparison with those produced from hand-picked grapes (50) can be explained by the significantly higher level of amino acids from mechanically harvested grapes, probably the result of longer contact with air and leaves, which increases the possibility of chemical and enzymatic reactions, in the must (50) e.g.

- that of the phenoloxidase, which can oxidize phenolic compounds to quinones, whiche in turn can oxidize amino acids to corresponding keto acids;
- an increased proteolitic grape activity because of mechanical processes, which increases the enzymatic transformation of soluble nitrogen (72).

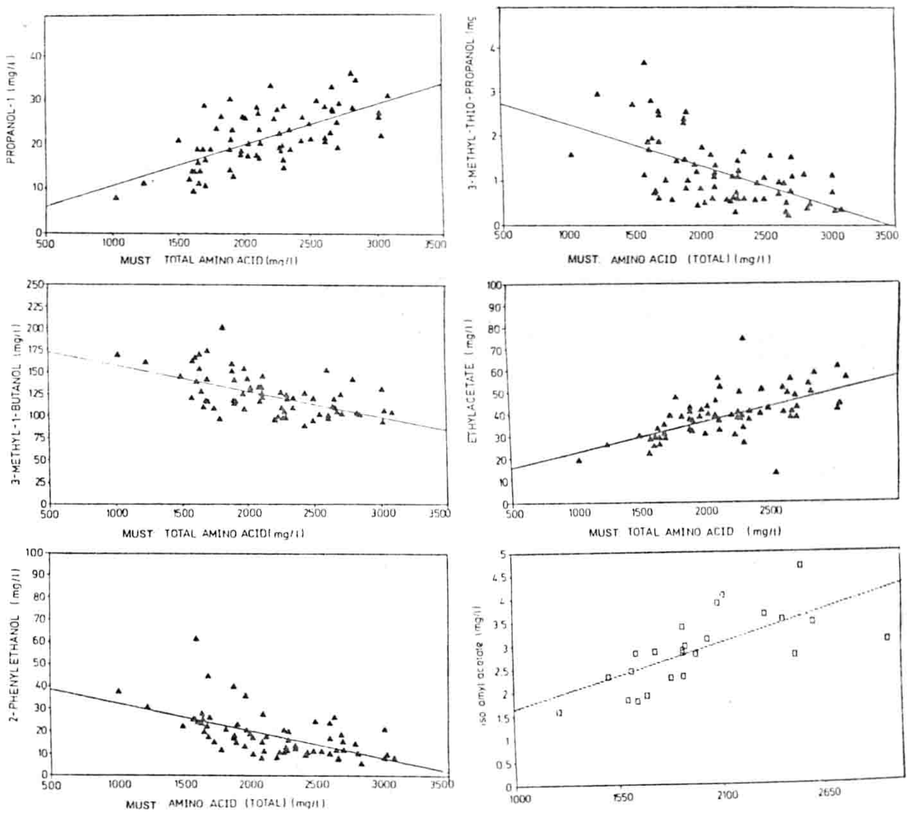

Fig. 7: Influence of total amino acids content in must and fermentation compounds in wine (Seeber, Versini et al., 51)

Such reaction mechanisms have opposite effects on the amino acid compositions of musts (50):
- decreases in amino acid levels because of their oxidation through quinones;
- increases in amino acid levels from increased protease activity.