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Use of an experimental design model to determine the impact of different fermentation parameters on the development of flavour compounds in wine

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Summary

An experimental design developed by Youden and Steiner (1975) was successfully applied to micro-fermentation experiments with two different grape musts. This tool allowed the verification of the impact of several fermentation parameters on the fermentation course and on flavour development with a restricted number of experiments. The positive effects of a higher fermentation temperature on the development of 3-mercaptohexanol, an important contributor to the characteristic aroma of the Petite Arvine wine, could be demonstrated.

K e y w o r d s : Micro-fermentation, vinification parameters, flavour development, experimental design.

Introduction

Thiol compounds, particularly 3-mercaptohexanol, have been shown to be of outstanding importance for the flavour of Sauvignon blanc wine (Darriet *et al.* 1995, Tominaga *et al.* 1998 a, Tominaga 1998). 3-Mercaptohexanol was also identified in Merlot and rosé wines made from Merlot grapes (Blanchard 2000; Murat *et al.* 2001 a), white wines from the Alsace (Tominaga *et al.* 2000 a) and Petite Arvine, an autochthone wine specialty produced in the Canton of Valais, Switzerland (Fretz *et al.* 2005). The precursors of the flavour active thiols are *S*-cysteine conjugates that are transformed into the free thiols during alcoholic fermentation by a yeast β-lyase (Tominaga *et al.* 1995, 1998 b). The transformation rates from the precursors have been shown to be low (Peyrot des Gachons 2000).

Peyrot des Gachons *et al.* (2000, 2002) studied the influence of pre-fermentation procedures on the content of the precursors of thiols that are important for the characteristic aroma of Sauvignon blanc. Maceration of grape berries led to a higher concentration of the cysteinylated precursor of the aroma active thiols, thereof the precursor of 3-mercaptohexanol increased by 50 %. This phenomenon is the consequence of the localization of the precursor in the skin of the berries (Peyrot des Gachons *et al.* 2002). The application of pectinolytic enzymes, however, did not increase the concentration of precursors, but decreased the concentration of thiols in the finished wines (Peyrot des Gachons 2000). Schneider *et al.* (2004) showed that wine aging "sur lies" increased the content of 3-mercaptohexanol, probably because of the reducing effects and the oxygen consumption of the yeast lees.

Youden and Steiner (1975) proposed a statistical method to verify the ruggedness of analytical methods and procedures based on experimental modelling design. This method allows to estimate the degree of influence of chosen parameters on the result of the analysis. The method has been applied, *e.g.* by Mirza and Tan (2001) to verify the robustness of newly developed analytical methods.

In this study,"Youden's procedure" was applied to study the influence of some vinification parameters on the transformation rate of the flavour precursor (*S*-cysteine conjugate) into 3-mercaptohexanol and on the formation of two typical fermentation products (2-phenylethanol and 2-phenylethyl acetate) during alcoholic fermentation. 3-Mercaptohexanol was determined because of the above mentioned importance as flavour impact compound of Petite Arvine and many other wine varieties. 2-Phenylethanol and 2-phenylethyl acetate are not impact compounds of Petite Arvine wine but general constituents of wine aroma and have been identified in numerous alcoholic beverages (Kieser *et al.* 1964, Schieberle 1994, Ribéreau-Gayon *et al.* 1998). These two substances were determined to verify the usefulness of the experimental model because of their general interest.

Material and Methods

E x p e r i m e n t a l m o d e l : "Youden's procedure" uses a combination of 7 parameters in 8 batches. To fulfil the prerequisites of this approach, each parameter had to be used in two variants, which were selected to have a high and a low value for each parameter in the model. If possible, two extreme values were taken (*e.g.* temperature of 16 °C and 25 °C, respectively); the variants did not need to be realistic in practice.

The following 7 parameters were studied: Turbidity of the must, yeast strain, temperature of the alcoholic fermentation, de-acidification of the must, suspension of the lees, presence of oxygen and nitrogen supplementation (Tab. 1). In the 8 fermentation batches, 4 times the high variant and 4 times the low variant of the parameter have to be applied. After fermentation, the concentrations of 3-mercaptohexanol, 2-phenylethanol and 2-phenylethyl acetate were

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Table 1

The experimental design with the two chosen variants

Parameter	Variant 1		Variant 2	
Turbidity	A	NTU 11	a	NTU 740-765
Yeast strain	B	*S. cerevisiae* VL1	b	*S. cerevisiae* CEPPO 20
Fermentation temperature	C	T = 16 °C	c	T = 25 °C
De-acidification	D	Total acidity 3.8 $g \cdot l^{-1}$	d	Total acidity 4.2 $g \cdot l^{-1}$
Suspension of the lees	E	With stirring	e	Without stirring
Oxidation	F	Headspace filled with air	f	Headspace filled with nitrogen
Nitrogen supplementation	G	No supplementation of ammonium sulfate	g	Supplementation of ammonium sulfate (0.3 $g \cdot l^{-1}$)

determined. The results of the different batches were assigned to the letters s-z (Tab. 2).

The influence of each parameter on the fermentation process is determined by calculating the mean value and the absolute differences between the two variants (Tab. 3). The higher the absolute difference, the higher the influence of this parameter on the result, *e.g.* on the amount of flavour compound developed during the fermentation. The algebraic sign (±) before calculating the absolute value indicates whether the variant with the capital letter (A-G) or with the lower case-letter (a-g) influences the result positively or negatively.

Table 2

Combination of the parameter variants in the different batches

Batch	Parameter							Results
1	A	B	C	D	E	F	G	s
2	A	B	c	D	e	f	g	t
3	A	b	C	d	E	f	g	u
4	A	b	c	d	e	F	G	v
5	a	B	C	d	e	F	g	w
6	a	B	c	d	E	f	G	x
7	a	b	C	D	e	f	G	y
8	a	b	c	D	E	F	g	z

Table 3

Calculation of the mean value and the absolute difference of each parameter

Mean value		Absolute difference
$\overline{A}=\frac{(s+t+u+v)}{4}$	$\overline{a}=\frac{(w+x+y+z)}{4}$	$Da=\lvert\overline{A}-\overline{a}\rvert$
$\overline{B}=\frac{(s+t+w+x)}{4}$	$\overline{b}=\frac{(u+v+y+z)}{4}$	$Db=\lvert\overline{B}-\overline{b}\rvert$
$\overline{C}=\frac{(s+u+w+y)}{4}$	$\overline{c}=\frac{(t+v+x+z)}{4}$	$Dc=\lvert\overline{C}-\overline{c}\rvert$
$\overline{D}=\frac{(s+t+y+z)}{4}$	$\overline{d}=\frac{(u+v+w+x)}{4}$	$Dd=\lvert\overline{D}-\overline{d}\rvert$
$\overline{E}=\frac{(s+u+x+z)}{4}$	$\overline{e}=\frac{(t+v+w+y)}{4}$	$De=\lvert\overline{E}-\overline{e}\rvert$
$\overline{F}=\frac{(s+v+w+z)}{4}$	$\overline{f}=\frac{(t+u+x+y)}{4}$	$Df=\lvert\overline{F}-\overline{f}\rvert$
$\overline{G}=\frac{(s+v+x+y)}{4}$	$\overline{g}=\frac{(t+u+w+z)}{4}$	$Dg=\lvert\overline{G}-\overline{g}\rvert$

The critical difference varies upon the assay; according to the Swiss Food Manual differences < 10 % of the mean value of the analysis results (s-z) have a minor influence (SWISS FOOD MANUAL 1993).

In order to estimate the natural variability between the concentrations of flavour compounds of two identically fermented batches, two batches were fermented under realistic conditions (20 °C, untreated must).

P a r a m e t e r s : The turbidity of the different fermentation batches was measured in NTU (Nephelometric Turbidity Units) by comparing the turbidity of the must before inoculation of the yeast with a reference solution of formazine (solution of hexamethylen tetramin and hydrazine sulphate (Fluka, Buchs, Switzerland) using the turbidity photometer LPT 4 (B. Lange, Berlin, Germany). The method was carried out as described in the SWISS FOOD MANUAL (1993). The turbidity of the must was adjusted with its own suspending matter deposited on the bottom of the containers. The used variants were NTU of 11 and NTU of 740-765.

Two different commercial *Saccharomyces cerevisiae* yeast strains were used in this study: *S. cerevisiae* Zymaflore VL1 (Laffort, Bordeaux, France) and *S. cerevisiae* CEPPO 20 (Littorale Oenologie, Langon, France). The yeast strains were re-hydrated and inoculated following to the manufacturer's instructions.

The fermentations were carried out at 16 °C and 25 °C respectively. In order to keep the temperatures constant, the fermentation took place in incubation stoves.

The total acidity of the must was lowered by addition of $CaCO_3$ to form insoluble tartaric acid complexes. The addition of 1 $g \cdot l^{-1}$ of $CaCO_3$ decreased the total acidity by 1.5 $g \cdot l^{-1}$ (calculated as tartaric acid). The deacidified must was separated from the insoluble particles by manual decanting. The total acidity of the must was determined by titration with 0.1 M NaOH, and calculated as tartaric acid (SWISS FOOD MANUAL 1993). The acidity of the batches was adjusted to 3.8 $g \cdot l^{-1}$ and 4.2 $g \cdot l^{-1}$ respectively. The acidity was low because of the prior refrigeration of the must and the consequent precipitation of tartaric acid.

The yeast lees were kept in suspension by stirring the fermentation flasks daily.

The contact of the grape must with oxygen was minimized by flushing the flasks with nitrogen (Pangas, Dagmersellen, Switzerland, quality 4.0) before filling and the headspace after filling. The other variant was not treated specially.

Nitrogen supplementation was made according to the Swiss Food Legislation (SCHWEIZERISCHE LEBENSMITTELVERORDNUNG 2002) which allows the addition of 0.3 $g \cdot l^{-1}$ ammonium sulphate (Fluka).

Fermentation: The fermentations were carried out in 2 l Erlenmeyer flasks with specially designed seals filled with glycerine in order to allow gas to escape but no gas to enter. All the batches were prepared, inoculated and incubated at the same time. The fermentation course was followed by measuring the weight loss due to CO_2 production. The sugar content was determined semi-quantitatively using the "Diabur-Test", which indicates the sugar concentration by coloration of a test strip (Roche Diagnostics, Rotkreuz, Switzerland).

Must of Chasselas grapes was used to study the development of 3-mercaptohexanol; the must was supplemented with 100.1 $\mu g \cdot l^{-1}$ of synthetic flavour precursor (*S*-3-(hexan-1-ol)-L-cysteine) (LUISIER *et al.,* submitted). For the studies on the development of 2-phenylethanol and 2-phenylethyl acetate, must of Petite Arvine grapes was used. The grape must was sulphated (50 $mg \cdot l^{-1}$) and stored at -20 °C until use.

Analysis of 3-mercaptohexanol: 3-Mercaptohexanol was extracted and its concentration determined according to the method developed and optimized by TOMINAGA *et al.* (1998 c, 2000 b). In this method, the thiols are selectively extracted by a complexation with *para*-hydroxymercuric benzoic acid (*p*HMB). The thiol compounds were analyzed by GC-FPD. The following chromatographic conditions were used:

Gaschromatograph:	HRGC, 5300 Mega Series (Carlo Erba Instruments, Milano, Italy)
Column:	BP 20 column (SGE, Melbourne, Australia); 50 m x 0.22 mm, ID 0.25 µm
Temperature program:	40 °C, 1 min; rate 3 °C min^{-1}; and 230 °C, 10 min
Injection volume:	3 µl (spiltless; split opened after 2 min)
Injector temperature:	240 °C
Detector:	FPD, supplemented with air (70 kPa) and hydrogen (150 kPa); 160 °C, bottom temperature 300 °C
Carrier gas:	Helium (100 kPa).
Data acquisition:	ChromCard, Version 1.07 (Thermo Quest, Milano, Italy).

All analyses were carried out in duplicate and the mean value was calculated.

Analysis of 2-phenylethanol and 2-phenylethyl acetate: The fermentation products 2-phenylethanol and 2-phenylethyl acetate were quantified by SPME extraction and GC analysis as described by RODRIGUEZ *et al.* (2002), but slightly modified: The wine sample (10 ml) and NaCl (2.9 g, Fluka) were placed in a 20 ml vial (BGB-Analytik, Adliswil, Switzerland). The volatiles present in the headspace of the vial were adsorbed by a PDMS 100 fibre (Supelco, Bellefonte, USA) at a sample temperature of 30 °C for 40 min. The volatiles were analyzed by GC-FID after desorption of the fibre in the splitless injector of the GC at 300 °C during 2 min. The GC (Agilent Technologies 6890 N, Palo Alto, USA) equipped with a DB-Wax column (30 m x 0.25 mm x 0.25 µm; J&W Scientific, Folsom, USA) was operated at the following conditions: Initial temperature 40 °C, 1 min. The temperature was raised to 230 °C at a rate of 5 °C·min^{-1}. Helium was used as carrier gas at a pressure of 1 ml·min^{-1}. The FID detector (300 °C) was supplemented with 40 ml·min^{-1} hydrogen and 400 ml·min^{-1} air. Quantification was done by using solutions of 2-phenylethanol (concentration range 5-200 $mg \cdot l^{-1}$) and 2-phenylethyl acetate (20-1000 $\mu g \cdot l^{-1}$) as external standards and a calibration curve (both substances from Fluka). All analyses were carried out in duplicate and the mean values were used for calculation.

Results and Discussion

Choice of the vinification parameters: The fermentation parameters were chosen as a function of their potential influence on yeast growth (fermentation course) and formation of aromatic compounds. Extreme variants of each parameter were chosen as proposed by YOUDEN and STEINER (1975). The variants did not need to be realistic in practice, rather they were chosen as extremes in order to better estimate their influence on the investigated value.

All experiments were made in must ready to ferment, so the influence of parameters prior to that stage, *e.g.* ripeness of berries, maceration and pressing of grapes could not be taken into consideration.

Insoluble grape material present in grape must is mainly composed of insoluble polysaccharides, unsaturated lipids, metal ions and proteins (ALEXANDRE *et al.* 1994). Wines made from very turbid must have a "heavier" aroma, are green and bitter in taste, also they are richer in phenols and more sensitive to oxidation (RIBÉREAU-GAYON *et al.* 1998). Phenols can react with thiols to form odourless complexes (CAPOZZI and MODENA 1974). On the other hand, the suspending matter is important for yeast growth, due to nutritional elements and due to a mechanical support of the yeast cells. Turbidity values between 60 and 200 NTU (Nephelometric Turbidity Units) are recommended in order to ensure an optimal fermentation course and to avoid aroma alteration (RIBÉREAU-

GAYON *et al.* 1998). The applied yeast strain can have an influence on wine flavour (PATEL and SHIBAMOTO 2002), however, in a critical review (THORNGATE 1998) doubted about the importance of the yeast strain on wine flavour, since the results of some studies are contradictory. The yeast strain *S. cerevisiae* Zymaflore VL1 is often used to ferment Petite Arvine grapes and, according to the wine producers, favours the formation of fruity flavours. *S. cerevisiae* CEPPO 20 is used to ferment Chasselas wines and is reported to form aromatical neutral wines. High temperatures are favourable for the growth of yeast cells (RIBÉREAU-GAYON *et al.* 1998), but the fermentation temperature also influences the formation of aroma: Temperatures above 20 °C are correlated with low ester and high fusel alcohol production (RIBÉREAU-GAYON 1978), whereas fermentation temperatures below 18 °C are correlated with a high and unwanted ester development. The fermentation of Petite Arvine wine is usually conducted at about 20 °C according to several producers from the Canton of Valais. Deacidification of the must is usually carried out for sensorial reasons, however yeast and bacterial growth is also dependent on the acidity of the must. If the lees are in suspension the contact surface of the organisms and the substrate is larger and higher yields in metabolites can be expected. The re-suspension ("Batonage") is often applied for fermentation and ripening in oak barrels. Thiols are sensitive to oxidation. DUBOURDIEU and LAVIGNE (1990) found a significant decrease in the intensity of odours caused by thiols when the must was hyperoxygenated or even in presence of oxygen. The addition of nitrogen in form of ammonium salts promotes yeast growth (RIBÉREAU-GAYON *et al.* 1998).

Fermentation course: All the batches were weighted daily in order to estimate the fermentation course. The total weight loss is listed in Tab. 4.

The results of the weight loss were also evaluated according to the "Youden procedure". With the results s-z, the absolute differences were calculated as described in the experimental part; the values are listed in Tab. 5.

Table 4

Weight loss during alcoholic fermentation of Chasselas and Petite Arvine must under different conditions

Batch number	Denomination of results	Petite Arvine (g per batch)	Chasselas (g per batch)
1	s	197.5	111.1
2	t	197.3	121.7
3	u	203.8	107.6
4	v	202.5	119.5
5	w	207.9	122.7
6	x	210.0	133.4
7	y	209.5	121.5
8	z	211.6	141.4
mean value		*205.0*	*122.4*
standard deviation		5.61	10.9
relative standard deviation		2.74	8.94
10 % of the mean value		20.5	12.2

Table 5

Absolute differences of the parameters, calculated on weight loss during fermentation

	Parameter	Petite Arvine	Chasselas
Da	Turbidity	9.5	**14.8**
Dc	Temperature	0.7	**13.3**
Dd	Desacidification	2.0	3[a]
Df	Oxygen	0.3	2.6[a]
De	Suspension of the lees	1.4[a]	2[a]
Dg	Nitrogen supplementation	0.3	1.9
Db	Yeast strain	3.7	0.3

[a] number was positive before calculating the absolute values; highlighted values exceed 10 % of the mean value.

The fermentation course of the experiment with Chasselas must was only significantly influenced by the turbidity and the incubation temperature; the other parameters were shown to play a minor role. At high NTUs and at 25 °C sugar was metabolized faster and the weight decreased more rapidly. During the fermentation of Petite Arvine, the batches did not differ significantly in weight loss.

Batches with a high turbidity lost more weight than those with low turbidity. Musts with high turbidity have an additional supply of nutrients, the solid particles enhance the distribution of the yeast and they help to eliminate CO_2 (RIBÉREAU-GAYON *et al.* 1975).

The positive effect of higher fermentation temperatures on yeast growth are well known (RIBÉREAU-GAYON *et al.* 1998); the closer the fermentation temperature to 30 °C, the higher the growth rate.

With regard to the total weight loss of Petite Arvine wine no parameter caused a difference exceeding 10 % of the mean value. This could be explained by the different duration of the fermentation. The must of Petite Arvine was fermented within 7 d. Therefor, the sugar content was in the range of 0-2 $g \cdot l^{-1}$, except for batches 1 and 3, where 10 $g \cdot l^{-1}$ were left. Chasselas must was subsequently fermented during 12 d, in order to assure the completion of the fermentation of all batches. The reason for the incomplete fermentation of batches 1 and 3 of the Petite Arvine essay could have been the fermentation temperature of 16 °C and, in addition, the variant with low turbidity.

RAPP (1988) has shown that the formation of 2-phenylethyl acetate is completed after 4-5 d, *e.g.* before the completion of alcoholic fermentation. The formation of 2-phenylethyl acetate and 2-phenylethanol are highly correlated (NYKÄNEN 1986), and the same behaviour can be expected for 2-phenylethanol. The incomplete fermentations should therefore not query the study.

The impact of temperature on the fermentation course of Chasselas must is illustrated in the Figure.

Influence of the fermentation parameters on the concentration of 3-mercaptohexanol: The concentration of 3-mercaptohexanol after 12 d of fermentation of Chasselas must was between

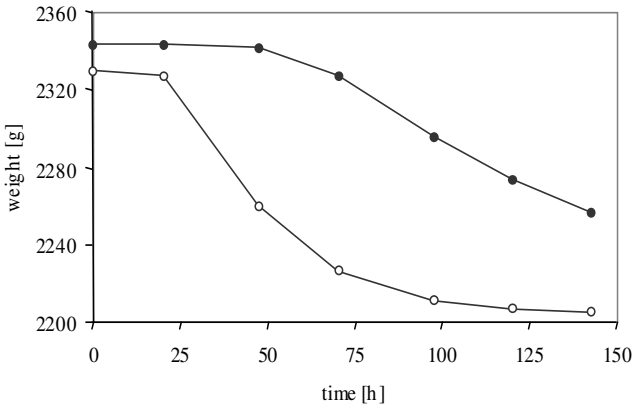

Figure: Influence of temperature (• 16 °C; o 25 °C) on the fermentation course of Chasselas must; mean values of 4 samples with the same fermentation temperature.

226 ng·l^{-1} and 430 ng·l^{-1} (Tab. 6), the highest and the lowest concentration differing by a factor of about 2. The concentrations of 3-mercaptohexanol are relatively small. Since 100.1 µg·l^{-1} (about. 80 % purity) were added, the transformation rate is between 0.37 and 0.71 % which is low compared to 2-10 % reported by PEYROT DES GACHONS (2000). The reasons remain to be elucidated. 3-Mercaptohexanol was quantified following the "YOUDEN procedure" (Tab. 7).

Table 6

Concentration of 3-mercaptohexanol in the 8 fermentation batches

Batch	Denomination of results	3-mercaptohexanol after fermentation (ng l^{-1})
1	s	230
2	t	311
3	u	216
4	v	430
5	w	226
6	x	381
7	y	279
8	z	354
mean value		303.5
standard deviation		79.4
relative standard deviation		26.2
10 % of the mean value		30.4

Temperature has been shown to be the most important parameter with respect to the formation of 3-mercaptohexanol during fermentation, high fermentation temperatures turning out to be favourable. The batch without nitrogen supplementation and the one using the yeast strain CEPPO 20 affected the formation of 3-mercaptohexanol positively as well, but to a less important degree than fermentation temperature. All the other parameters do not play a role regarding the release of 3-mercaptohexanol. The fact that high fermentation temperature affects the release of 3-mercaptohexanol positively is quite surprising. Lower fermentation temperatures are generally favourable for the fruity aroma of wines, mainly caused by alcohols and esters (OUGH and AMERINE 1967, RIBÉREAU-GAYON 1975). It might also be expected that reactions of the thiol (e.g. oxidations) would be accelerated at higher temperatures. To our knowledge, the characteristics of β-lyase of yeast have not been investigated yet, so no data could be found on temperature optima. It could be that the enzyme activity is increased at higher temperatures to such an extent, that the negative effects are outweighed. Nitrogen supplementation has a negative effect on the final 3-mercaptohexanol concentration. The yeast strain CEPPO 20 is not used in practice for the fermentation of Petite Arvine musts, although our results showed a higher formation rate for 3-mercaptohexanol than with the strain VL1. This should be verified at a real wine making scale.

Table 7

Absolute differences of the parameters, calculation based on the measured concentration of 3-mercaptohexanol

	Parameter	Absolute difference
Dc	Temperature	**131**
Dg	Nitrogen supplementation	**53.5**[a]
Db	Yeast strain	**32.5**
Dd	Deacidification	19.5
De	Suspension of the lees	16
Df	Oxidation	13.5[a]
Da	Turbidity	13

[a] number was positive before calculating the absolute values; highlighted values exceed 10% of the mean value.

The relative standard deviation for two identically fermented samples (12.0 %, data not shown) was lower than the relative standard deviation of the 8 batches in this trial (26.2 %). This indicates that the different variants influenced the concentration of 3-mercaptohexanol during the alcoholic fermentation.

Influence of the fermentation parameters on the concentration of 2-phenylethanol and 2-phenylethyl acetate: The concentration of two typical fermentation compounds (2-phenylethanol and 2-phenylethyl acetate) was also determined in each batch in order to verify the method (Tab. 8).

The concentrations of 2-phenylethanol and particularly of 2-phenylethyl acetate found are high compared to the contents in wines. However, it is known that the content of esters and alcohols are highest immediately after fermentation and are decreasing during ripening and storage of wine (BAYONOVE *et al.* 1998)

The relative standard deviations of two identically fermented samples were 9.2 % for 2-phenylethanol and 5.8 % for 2-phenylethyl acetate respectively (data not shown). This is lower than the relative standard deviation of the 8 samples (16.6, resp. 16.7 %), so it can be concluded that the different variants of the parameters had an influence on the concentrations. The calculations according to YOUDEN and STEINER (1975) are shown in Tab. 9.

Table 8

Concentration of 2-phenylethanol and 2-phenylethyl acetate in the 8 fermentation batches

Batch	Analysis result	2-Phenylethanol ($mg \cdot l^{-1}$)	2-Phenylethyl acetate ($\mu g \cdot l^{-1}$)
1	s	12.6	653
2	t	15.5	646
3	u	19.7	685
4	v	18.2	727
5	w	15.2	624
6	x	19.8	938
7	y	16.7	577
8	z	21.3	823
mean value		*17.4*	*709*
standard deviation		2.9	118
variant coefficient		16.7	16.6
10 % of the mean value		1.74	70.9

Table 9

Absolute differences of the parameters, calculation based on the concentrations of 2-phenylethanol and 2-phenylethyl acetate after fermentation

	Parameter	2-Phenyl ethanol	2-Phenylethyl acetate
Db	Yeast strain	**3.2**	12.25[a]
Dc	Temperature	**2.7**	**148.8**
De	Suspension of the lees	**2.0**[a]	**131.3**[a]
Da	Turbidity	**1.8**	62.8
Dd	Deacidification	1.7	68.8
Df	Oxidation	1.1	4.8
Dg	Nitrogen supplementation	1.1	29.3[a]

[a] number was positive before calculating the absolute values; highlighted values exceed 10 % of the mean value.

The fermentation temperature influences the formation of 2-phenylethanol and 2-phenylethyl acetate, with yeast strain CEPPO 20; more 2-phenylethanol was produced and the regular resuspending of the yeast lees favoured the formation of 2-phenylethyl acetate. WAGNER and RAPP (1999) also found a higher 2-phenylethanol production at higher fermentation temperatures. They also demonstrated that the yeast plays a role for the formation of 2-phenylethanol. Turbidity did not influence the formation of these fermentation products; these results agree with those of KANAGIANNIS and LANARIDIS (2002), showing no relation between must turbidity and the formation of 2-phenylethyl acetate. In a recent study HERNANDEZ-ORTE *et al.* (2005) report a significant decrease in phenylethanol when ammonium was added to the must; this is in contradiction with our findings.

Conclusion

Using the experimental design procedure for micro-vinification it was clearly demonstrated that the formation of 3-mercaptohexanol is more sensitive to the fermentation parameters than 2-phenylethanol and 2-phenylethyl acetate. High fermentation temperatures are particularly favourable for the release of 3-mercaptohexanol. The results obtained with the vinification trials confirm previous studies, thus proving the correctness of the experimental design. The method proposed by YOUDEN and STEINER (1975) is not only a powerful tool for studying the influence of a fermentation parameter on distinct quality aspects, as e.g. the concentration of a flavour compound, but also for the relevance of the parameter.

In the present study the results of the "YOUDEN analysis" do not deliver absolute results but tendencies, since extreme values of parameters were chosen. In practice, the development of one single aroma compound should be put in relation to the influence of other quality aspects, and a compromise has to be found. Besides aroma analysis, other applications of the micro-vinification procedure can be imagined. Using the same experimental design a scale up to the dimensions of a wine cellar should be carried out.

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References

ALEXANDRE, H.; NGUYAN VAN LONG, T.; FEUILLAT, M.; CHARPENTIER, C.; 1994: Contribution à l'étude des bourbes: Influence sur la fermentescibilité des fûts. Rev. Fr. Oenol. **145**, 11-20.

BAYONOVE, C.; BAUMES, R.; CROUZET, I.; GÜNATA. Z.; 1998: Arômes. In: C. FLANZY (Ed.): Oenologie, Fondements Scientifiques et Technologiques, 163-235. Lavoisier, Tec & Doc. London, Paris, New York.

BLANCHARD, L.; 2000: Recherche sur la contribution de certains thiols volatils à l'arôme des vins rouges. Etude de leur genèse et de leur stabilité. PhD Thesis Nr. 773. Univ. Victor Segalen Bordeaux II.

CAPOZZI, G.; MODENA, G.; 1974: Oxidation of thiols. In: S. PATAI (Ed.): The Chemistry of the Thiol Group, 785-839. Wiley, London.

DARRIET, P.; TOMINAGA, T.; LAVIGNE, V.; BOIDRON, J. N.; DUBOURDIEU, D.; 1995: Identification of a powerful aromatic component of *Vitis vinifera* L.var. Sauvignon wines: 4-Mercapto-4-methylpentan-2-one. Flavour Fragr. J. **10**, 385-392.

DUBOURDIEU, D.; LAVIGNE, V.; 1990: Incidence de l'hyperoxygénation sur la composition chimique et les qualités organoleptiques des vins blancs secs du bordelais. Rev. Fr. Oenol. **124**, 58-61.

FRETZ, C; LUISIER, J. L., TOMINAGA,T.; AMADÒ, R.; 2005: 3-mercatpohexanol, an aroma impact compound of a wine produced from an autochthone grape variety. Am. J. Enol. Vitic. (in press).

HERNANDEZ-ORTE, P.; IBARZ, M. J.; CACHO, J.; FERREIRA, V.; 2005: Effect of the addition of ammonium and amino acids to musts of Airen variety on aromatic composition and sensory properties on the obtained wine. Food Chem. **89**, 163-174.

KARAGIANNIS, S.; LANARIDIS, P.; 2002: Insoluble grape material present in must affects the overall fermentation aroma of dry white

wines made from three grape cultivars cultivated in Greece. J. Food Sci. **67**, 369-375.

KIESER, M. E.; POLLARD, A.; STEVENS, P. M.; TUCKNOTT O. G.; 1964: Determination of 2-phenylethanol in cider. Nature **204**, 887.

LUISIER, J. L.; PIANTINI, U.; VEYRAND, J.; A simple synthesis of S-3-(hexan-1-ol)-L-cysteine (submitted).

MIRZA, T.; TAN, H. S. I.; 2001: Determination of captopril in pharmaceutical tables by anion-exchange HPLC using indirect photometric detection; a study in systematic method development. J. Pharmaceut. Biomed. **25**, 39-52.

MURAT, M. L.; TOMINAGA, T.; DUBOURDIEU, D.; 2001 a: Assessing the aromatic potential of Cabernet Sauvignon and Merlot musts used to produce rose wine by assaying the cysteinylated precursor of 3-mercaptohexan-1-ol. J. Agric. Food Chem. **49**, 5412-5417.

NYKÄNEN, L.; 1986: Formation and occurrence of flavor compounds in wine and distilled alcoholic beverages. Am. J. Enol. Vitic. **37**, 84-96.

OUGH, C. S.; AMERINE, M. A.; 1967: Studies with controlled fermentation temperature on some volatile compounds in wine. Am. J. Enol. Vitic. **18**, 157-164.

PATEL, S.; SHIBAMOTO, T.; 2002: Effect of different strains of *Saccharomyces cerevisiae* on production of volatiles in Napa Gamay wine and petite Sirah wine. J. Agric. Food Chem. **50**, 5649-5653.

PEYROT DES GACHONS, C.; 2000: Recherches sur le potentiel aromatique des raisins de *Vitis vinifera* L. cv. Sauvignon blanc. PhD Thesis Nr. 799. Univ. Victor Segalen Bordeaux II.

PEYROT DES GACHONS, C.; TOMINAGA, T.; DUBOURDIEU, D.; 2002: Localisation of S-cysteine conjugates in the berry: effects of skin contact on aromatic potential of *Vitis vinifera* L. cv. Sauvignon blanc. Am. J. Enol. Vitic. **53**, 144-146.

RAPP, A.; 1988: Wine aroma substances from gas chromatographic analysis. In Modern methods of plant analysis, new series vol. 6. In: H. F. LINKENS; J. F. JACKSON (Eds.): Wine Analysis, 29-66. Springer Verlag, Berlin, Heidelberg, New York, London, Paris, Tokyo, 29-66.

RIBEREAU-GAYON, P.; 1978: Wine Aroma, In: G. CHALAMBOUS, G. E. INGLETT (Eds.): Flavor of Food and Beverages-Chemistry and Technology, 362-380. Academic Press, New York, San Francisco, London.

RIBÉREAU-GAYON, P.; DUBOURDIEU, D.; DONÉCHE, B.; LONVAUD, A.; 1998: Traité d'Oenologie, tome 1 and 2. Dunod, Paris.

RIBEREAU-GAYON, P.; LAFON-LAFOUCADE, S.; BERTRAND, A.; 1975: Racking of white wine grape musts. Conn. Vigne Vin **9**, 117-139.

RODRIGUEZ-BENECOMO, J. J.; CONDE, J. E.; RODRIGUEZ-DELGADO, M. A.; GARCIA-MONTELONGO, F.; PEREZ-TRUJILLO, J. P.; 2002: Determination of esters in dry and sweet white wines by headspace solid-phase microextraction and gas chromatography. J Chromatogr. A **963**, 213-223.

SCHNEIDER, R.; CHARRIER, F.; BAUMES, R.; 2004: Effet de la maturité des raisin et de l'élevage des vins sur lies sur le teneurs de quelques composes volatils caractéristiques des vins blancs de muscadet. Proc. 7. Int. Symp. Innovationen der Kellerwirtschaft, Stuttgart, 10-11. Mai 2004.

SCHIEBERLE, P.; 1994: Primary odorants of pale lager beer-differences to other beers and changes during storage. Z. Lebensm. Unters. Forsch. **193**, 558-565.

SCHWEIZERISCHE LEBENSMITTELVERORDNUNG; 2002:Verordnung des EDI über die zulässigen oenologischen Verfahren und Behandlungen.

SWISS FOOD MANUAL; 1993: Chapters 27, 30, 60

THORNGATE, J. H; 1998: Yeast strain and wine flavor: Nature or Nurture? In: A. L. WATERHOUSE; S. E. EBELER (Eds.): Chemistry of Wine Flavour, 66-80. ACS Symp. Series 714, ACS, Washington DC.

TOMINAGA, T.; 1998: Recherches sur l'arôme variétal des vins de *Vitis vinifera* L. cv. Sauvignon blanc et sa genèse à partir de précurseurs inodores du raisin. PhD Thesis Nr. 599. Univ. Victor Segalen Bordeaux II.

TOMINAGA, T.; BALTENWECK-GUYOT, R.; PEYROT DES GACHONS, C.; DUBOURDIEU, D.; 2000 a: Contribution of volatile thiols to the aromas of white wines made from several *Vitis vinifera* grape varieties. Am. J. Enol. Vitic. **51**, 178-181.

TOMINAGA, T.; BLANCHARD, L.; DARRIET, P.; DUBOURDIEU, D.; 2000 b: A powerful aromatic volatile thiol, 2-furanmethanethiol, exhibiting roast coffee aroma in wines made from several *Vitis vinifera* grape varieties. J. Agric. Food Chem. **48**, 1799-1802.

TOMINAGA, T.; FURRER, A.; HENRY, R.; DUBOURDIEU. D.; 1998a: Identification of new volatile thiols in the aroma of *Vitis vinifera* L. var. Sauvignon blanc wines. Flavour Fragr. J. **13**, 159-162.

TOMINAGA, T.; MASNEUF, I.; DUBOURDIEU, D.; 1995: Mise en évidence d'un S-conjugué de la cysteine, précurseur d'arôme du Sauvignon. J. Int. Sci. Vigne Vin **29**, 227-232.

TOMINAGA, T.; MURAT, M. L.; DUBOURDIEU, D.; 1998 c: Development of a method for analyzing the volatile thiols involved in the characteristic aroma of wines made from *Vitis vinifera* L. cv. Sauvignon blanc. J. Agric. Food Chem. **46**, 1044-1048.

TOMINAGA, T.; PEYROT DES GACHONS, C.; DUBOURDIEU, D.; 1998 b: A new type of flavor precursors in *Vitis vinifera* L. cv. Sauvignon blanc: *S*-Cysteine conjugates. J. Agric. Food Chem. **46**, 5215-5219.

WAGNER, K.; RAPP, A.; 1999: Über den Einfluss der Hefe auf die Bildung von 2-Phenylethanol bei der alkoholischen Gärung. Deut. Lebensm. Rundsch. **8**, 304-309.

YOUDEN, W. J.; STEINER E. H.; 1975: Ruggedness Test for Procedures. Statistical Manual of the AOAC, 33-36.

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