The effect of Yeast Strain, Grape Solids, Nitrogen and Temperature on Fermentation Rate and Wine Quality[1)]

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The effect of four yeast strains, two levels of grape solids, two nitrogen levels and three fermentation temperatures viz. 10°, 13° and 15° C on total fermentation time (TFT) and wine quality was studied in a factorial experiment by employing a nitrogen deficient Chenin blanc must. It was found that the presence of solids, addition of nitrogen and fermenting at 15° C decreased TFT dramatically. Nitrogen was the only factor which also increased wine quality, the other two having a detrimental effect. At optimum levels of nitrogen, yeast strains WE 452 and WE 500 (VORI collection) reduced TFT without detrimentally affecting wine quality. In the case of WE 452, no solids should be present and fermentation conducted at 10° C — 13° C, and in the case of WE 500, traces of solids should be present and a fermentation temperature of between 13° C — 15° C should be utilized. Under these conditions WE 452 and WE 500 reduce TFT from C.76 days to as few as C.30 and C.15 days respectively.

It has been established that several factors contribute to the occurrence of stuck or lagging fermentations. Low levels of nitrogen (Agenbach, 1977; Vos, Zeeman & Heymann, 1978; Van Rooyen & Tromp, 1982) and absence or low levels of grape solids (Groat & Ough, 1978; Houtman & Du Plessis, 1981; Van Rooyen & Tromp, 1982) seem to be the two main factors in this respect. Although oxygen is required for sterol synthesis (Aries & Kirsop, 1978) which is necessary to complete fermentation (Larue, Lafon-Lafourcade & Ribéreau-Gayon, 1979; Strydom, Kirschbaum & Tromp, 1982), its addition to must is detrimental to wine quality (Tromp, 1980; Houtman & Du Plessis, 1981). The latter authors also pointed out that the oxygen concentration of musts is sufficiently high (C.8 mg.ℓ^{-1}) under practical conditions for fermentations to proceed to dryness. Low temperatures also retards fermentation. Ough (1966) reported a 50% reduction in fermentation time when fermentation temperature was increased from 10° C to 21° C. Van Rooyen & Tromp (1982) similarly found that fermentation took 14,6 days on average at 20° C as against 20,6 days at 13° C.

It was found by Groat & Ough (1978) that bentonite or talc addition to clear must stimulated fermentation but Van Rooyen & Tromp (1982) could not confirm these results. This was probably due to the yeast strain which was employed in the latter study. The strain was WE 14 from the VORI collection and, being a bottom fermentor, was probably flocculated from suspension by the added bentonite.

The purpose of this study was to investigate the effect of grape solids, nitrogen addition and temperature of fermentation on the performance of different yeast strains, primarily with regard to TFT and the quality of the resultant wines.

MATERIALS AND METHODS

Must Treatments: Must from a Chenin blanc vineyard, known to be prone to stuck or lagging fermentation, mainly because of a nitrogen deficiency (Agenbach, 1977; Van Rooyen & Tromp, 1982), was used in this study. The analysis of the must was as follows:

Reducing sugar	=	225 g.ℓ^{-1} (as invert sugar)
Fixed acidity	=	9,8 g.ℓ^{-1} (as tartaric acid)
pH	=	3,08
Amino nitrogen	=	98 mg.ℓ^{-1} (as lysine) (Anon., 1973)
Total nitrogen	=	270 mg.ℓ^{-1} (as N).

The must was subdivided into 48 duplicate fermentations in 20ℓ stainless steel containers and sulphur dioxide added to 50 mg.ℓ^{-1}. Using a factorial design the following treatments were applied:

— Two levels of grape solids viz:

no solids (S_1) — obtained by filtration of settled juice; traces of solids (S_2) — obtained by clear settling of the juice (although slightly turbid, no grape solids could be determined by centrifugation).

— Two levels of nitrogen viz:

270 and 520 mgN.ℓ^{-1} must (N_1 and N_2 respectively. Adjusted by the addition of $(NH_4)_2$ HPO_4).

— Four yeast strains viz: WE 452, WE 500, WE 14 and WE 432 of the VORI collection.

— Three fermentation temperatures viz: 10° C, 13° C and 15° C.

Inoculations of 5 × 10^6 yeast cells/ mℓ must were made by adding the rehydrated dried yeast directly to each stainless steel container. Fermentation rates were recorded by daily mass determinations and periodic sugar analyses towards the end of fermentation. At dryness TFT was noted and the wines filtered and cold stabilized (0° C). Those wines which were still fermenting 76 days

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after inoculation were also filtered and cold stabilized. After a second filtration and bottling all the wines were stored at 0° C to prevent any changes in the quality and/or composition of the wines. The wines were subsequently analysed for: Density (20° C), alcohol (vol. %), extract ($g.\ell^{-1}$), total reducing sugars ($g.\ell^{-1}$), volatile acidity ($g.\ell^{-1}$) and free and total sulphur dioxide by employing methods of Anon. (1975).

Wine quality (overall impression as well as aroma) was determined by employing the 9-point scorecard as discussed by Tromp & Conradie (1979) utilizing a panel of 12 expert judges. The scores were expressed as a percentage.

Data Processing: An analysis of variance was performed on the data and use was also made of the Scott-Knott method of grouping means in a cluster analysis (Scott & Knott, 1974).

RESULTS AND DISCUSSION

Raw data: To give an idea of the magnitudes and variation within the data set a summary of analytical results and quality ratings is given in Table 1.

TABLE 1
Magnitude and variation of analytical and sensory data obtained in this study.

Variable	Mean	Minimum	Maximum	Standard deviation
Total ferm. time (days)	51	12	76	5,8
Density (20° C)	0,99205	0,99059	0,99770	0,00047
Alcohol (vol. %)	13,25	12,51	13,57	0,14
Extract ($g.\ell^{-1}$)	24	21	38	1
Sugar ($g.\ell^{-1}$)	3,5	1,1	15,4	1,3
Total acidity ($g.\ell^{-1}$)	7,55	7,10	7,95	0,14
Volatile acidity ($g.\ell^{-1}$)	0,43	0,18	0,59	0,04
pH	3,07	2,86	3,29	0,05
SO_2 (free, $mg.\ell^{-1}$)	40	24	50	6
SO_2 (total, $mg.\ell^{-1}$)	90	55	116	10
% Free SO_2	45	17	63	9
Overall quality (%)	51,4	31,4	63,0	3,8
Aroma quality (%)	51,3	32,4	63,0	3,2

As can be seen in Table 1 TFT varied from 76 to 12 days, and the overall quality of the resultant wines from 31,4% to 63,0% in response to the treatments mentioned before.

In Table 2 the composite means of the different parameters measured are shown. Data of density, alcohol and extract was omitted as some wines did not ferment to dryness. Percentage free sulphur dioxide (of total sulphur dioxide) was calculated from and given in place of free and total sulphur dioxide.

Main effects: The main effects of the different treatments on TFT can be seen in Table 2. It is evident that all the treatments had a significant effect on TFT. The presence of grape solids, even in such small quantities that it could not be determined by centrifugation, shortened TFT from 62,4 to 40,3 days. This is in accordance with the studies of Groat & Ough (1978) and Van Rooyen & Tromp (1982). The addition of nitrogen also decreased TFT from 56,3 to 46,4 days on average as was expected and previously pointed out by Agenbach (1977) on must from the same vineyard in that year.

The yeast strain employed had a marked effect on TFT, WE 452 completing fermentations at 33,6 days on average, while WE 500, WE 432 and WE 14 respectively took 47,1, 59,3 and 65,5 days to do the same.

In the case of fermentation temperature the well-known fact that fermentation is slower at lower temperatures was established once more; under the same set of conditions fermentation at 15° C, 13° C and 10° C caused TFT's of respectively 38,0, 54,4 and 61,6 days on average.

From the above it should follow that to obtain the shortest TFT in terms of the treatments applied, traces of

TABLE 2
Wine parameter data as affected by several treatments of a Chenin blanc must

Treatments	Wine parameters							
	TFT (days)	Sugar ($g.\ell^{-1}$)	Total acidity ($g.\ell^{-1}$)	Volatile acidity ($g.\ell^{-1}$)	pH	Overall quality (%)	Aroma quality (%)	Percentage Free SO_2
	*	*				*	*	
No grape solids	62,4 A	5,0 A	7,53	0,48	3,09	54,3 A	55,0 A	46
Traces of grape solids	40,3 B	2,1 B	7,56	0,38	3,05	47,6 B	47,7 B	45
	**	**	**	**	*	**	*	
No nitrogen	56,3 A	4,1 A	7,39 B	0,46 A	3,11 A	48,4 B	48,3 B	45
Addition of nitrogen	46,4 B	2,9 B	7,70 A	0,40 B	3,03 B	54,5 A	54,3 A	47
	**	**	**	**	**	**	**	**
Yeast strain WE 452	33,6 D	1,5 D	7,45 D	0,50 A	3,01 C	48,7 B	49,6 C	37 D
Yeast strain WE 500	47,1 C	4,7 B	7,55 B	0,43 C	3,07 B	53,4 A	53,9 A	48 B
Yeast strain WE 14	65,5 A	5,0 A	7,50 C	0,46 B	3,13 A	54,2 A	52,6 B	44 C
Yeast strain WE 432	59,3 B	2,9 C	7,69 A	0,33 D	3,06 B	49,4 B	49,3 C	54 A
	**	**		**	**	**	**	
15°C	38,0 C	2,1 C	7,53	0,40 C	3,04 C	49,5 C	47,9 B	44
13°C	54,4 B	3,4 B	7,56	0,42 B	3,07 B	51,9 B	52,9 A	45
10°C	61,6 A	5,1 A	7,56	0,46 A	3,09 A	52,9 A	53,2 A	48
Average	51,34	3,52	7,55	0,43	3,07	51,1	51,3	45,8

(1) *and** denotes significance at levels $P \leqslant 0,05$ and $P \leqslant 0,01$ respectively
(2) The letters A-C designates groups which are significantly different according to Scott & Knott (1974)

grape solids should be present in the must, nitrogen should be added, WE 452 should be employed as yeast strain and fermentation should proceed at a temperature of 15° C. It was in fact found in this experiment that application of these conditions yielded the shortest TFT, namely 12 days. The longest TFT should (Table 2) be encountered when using filtered must to which no nitrogen is added while fermentation is conducted with yeast strain WE 14 at 10° C. This was found to be the case —these conditions caused a TFT of 76 days — at which time all those fermentations which had not proceeded to dryness were stopped. The fermentation curves for the two above-mentioned fermentations are given in Fig. 1.

measured. Although differences occurred between yeast strains as far as TA and pH were concerned these were of no practical significance. WE 452 formed more VA than did any of the other strains (0,5 g.ℓ^{-1}) while WE 500 formed less (0,43 g.ℓ^{-1}) than either WE 452 or WE 14 (0,5 and 0,46 g.ℓ^{-1}). It is also apparent that WE 452 forms more SO_2-binding substances such as aldehydes than the other yeast strains — the % free SO_2 being markedly less than in the case of WE 500 (48%) and WE 432 (54%). This would mean that wines fermented with WE 452 will require a higher concentration of total sulphur dioxide than the other yeast strains to retain the same amount of free SO_2 in the wine.

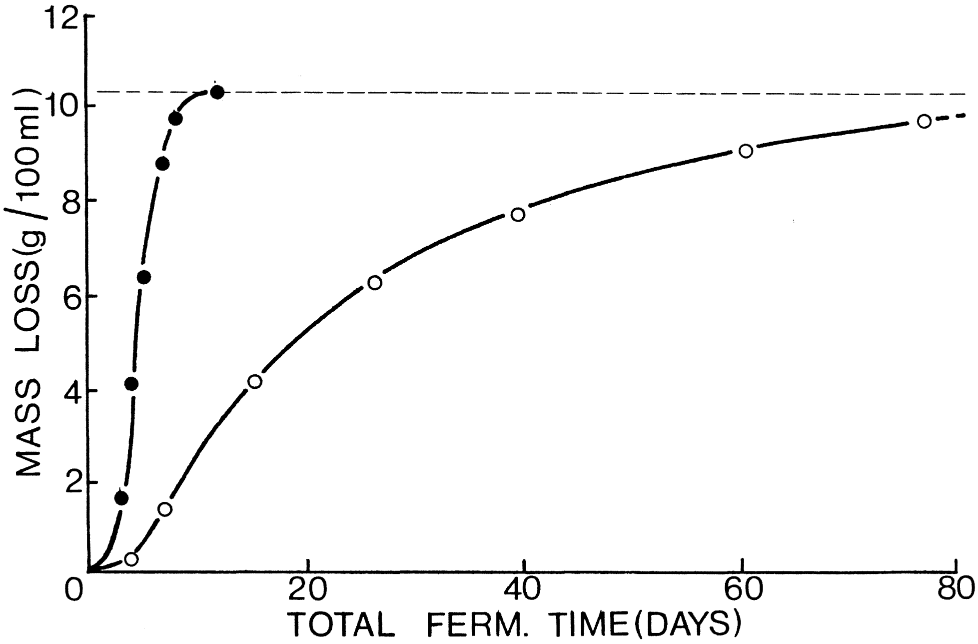

FIGURE 1
Fermentation curves in a Chenin blanc must of yeast strains WE 452 (in the presence of solids, with addition of nitrogen at 15° C-●) and WE 14 (in filtered must with no nitrogen added at 10° C-○).

All the treatments had a significant effect on quality (Table 2). As in previous research (Houtman & Du Plessis, 1981; Van Rooyen & Tromp, 1982) the presence of grape solids had a negative effect on wine quality (47,6% as against 54,3% where solids were absent). The addition of nitrogen increased wine quality from 48,4% to 54,5% on average. Note that the addition of nitrogen increased both fermentation rate and wine quality. This was also found by Vos, Zeeman & Heymann (1978) and Van Rooyen & Tromp (1982).

The yeast strain employed also had a marked effect on wine quality, WE 500 and WE 14 yielding wines of higher quality than those fermented with WE 452 and WE 432 (53,4% and 54,2% as against 48,7% and 49,4% respectively). Lower temperatures increased wine quality (52,9%, 51,9% and 49,5% for 10° C, 13° C and 15° C respectively).

The effect of the different treatments on the total —and volatile acidity concentrations (TA & VA) as well as on pH and % free sulphur dioxide will now be discussed in brief (Table 2).

The presence or absence of grape solids had no significant effect on these measurements. The addition of nitrogen, however, caused a higher TA in the resultant wines with pH and VA being lower. While lower fermentation temperature only caused higher VA and pH, the yeast strain employed had an effect on all four parameters.

Interactions affecting TFT and wine quality: The main interaction which occurred affecting both TFT and quality was between the presence of solids and addition of nitrogen (Table 3).

TABLE 3
Composite means for total fermentation time (TFT) and wine quality as affected by grape solids and nitrogen.

Grape Solids	No nitrogen added		Nitrogen added	
	TFT (days)	Qual. (%)	TFT (days)	Qual. (%)
None	65,5	54,3	59,3	56,2
Traces	47,1	42,6	33,6	52,9

LSD (TFT) P$\leqslant$ 0,01 = 2 days

LSD (Quality) P$\leqslant$ 0,01 = 3,6%

P$\leqslant$ 0,05 = 2,8%

Calculated for the solids x nitrogen interaction.

In the absence of solids TFT was marginally decreased from 65,5 to 59,3 days by the addition of nitrogen, while wine quality was virtually unaffected. The presence of solids reduced TFT whether nitrogen was added or not. At the lower nitrogen level wine quality, however, was severely impaired by the presence of solids (42,6% as against 54,3%), while only a slight, but significant loss in quality was encountered when solids were present at the higher level of nitrogen (52,9% as against 56,2%). What is however of greater importance is that the presence of only traces of grape solids decreased TFT from 65,5 to 33,6 days on average when also increasing the nitrogen level, while wine quality was not at all affected (54,3% as against 52,9%). These results are the average for four yeast strains and are in accordance with the results obtained by Tromp (1980) where the same relationship between presence of solids and nitrogen addition was shown for only one yeast strain (WE 14). This interaction cannot be explained at present.

This relationship (S × N) was somewhat different for the different yeast stains. In Fig. 2 the effect of grape solids and nitrogen addition on TFT (Fig. 2A) and wine quality (Fig. 2B) is given for the different yeast strains in a three-dimensional outlay. When no solids were present or no nitrogen was added (S_1N_1, Fig. 2A), WE 452 had a TFT of 50,5 days which was significantly shorter than the 73,7, 70,7 and 67,0 days for WE 500, WE 14 and WE 432 respectively. When no solids were present but nitrogen was added (S_1N_2), TFT was drastically reduced for WE 452 (to 27,5 days), somewhat reduced for WE 500 (to 68,7 days) but stayed the same for WE 14 and WE 432, although the residual reducing sugars were slightly less where nitrogen was added (see numbers in brackets, Fig. 2A).

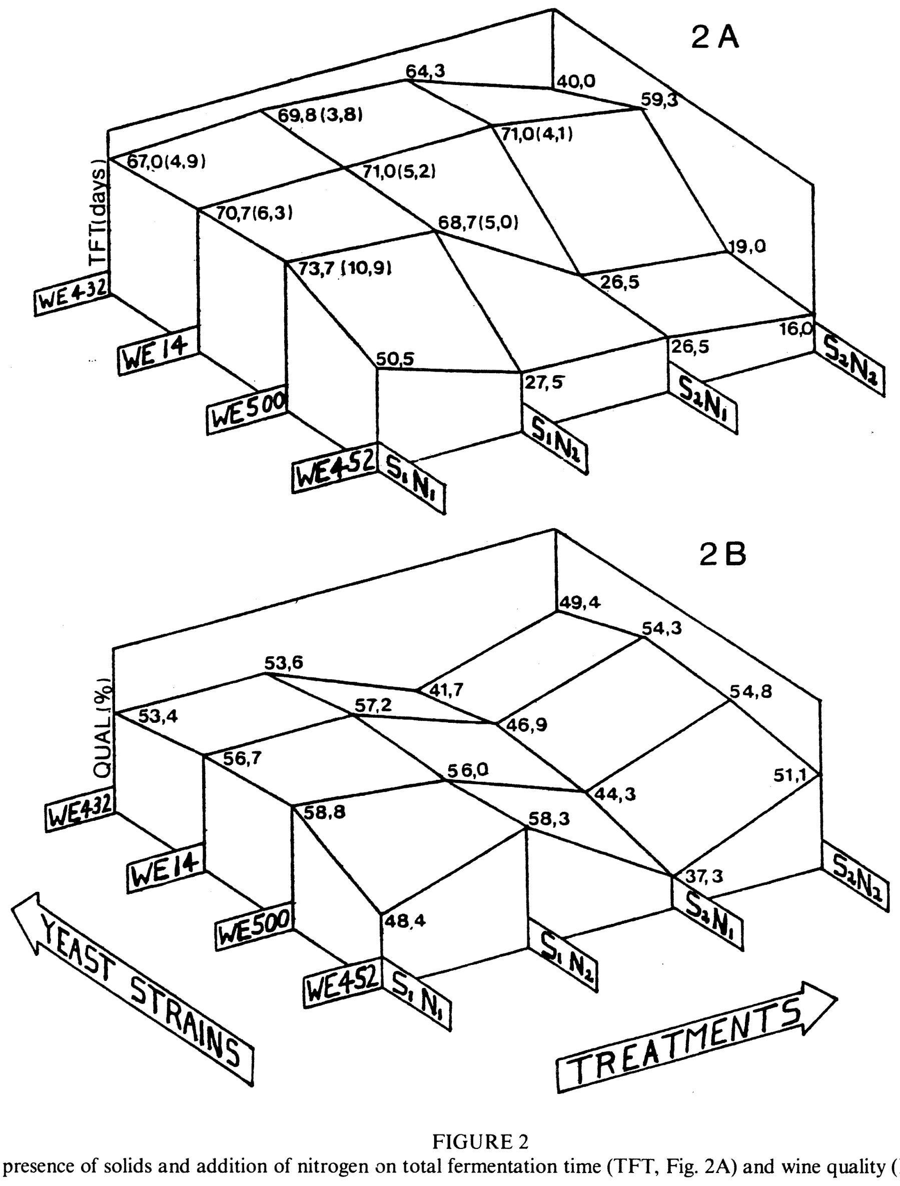

FIGURE 2

Effect of yeast strain, presence of solids and addition of nitrogen on total fermentation time (TFT, Fig. 2A) and wine quality (Fig. 2B).

S_1 = no solids
S_2 = traces of solids
N_1 = no nitrogen
N_2 = addition of nitrogen.

LSD (TFT) $P \leqslant 0,05 = 4,4$ days
$P \leqslant 0,01 = 5,2$ days
LSD (Qual). $P \leqslant 0,05 = 7,7\%$
$P \leqslant 0,01 = 8,8\%$

Calculated for the yeast strain x solids x nitrogen interaction

Numbers in brackets in Fig. 2A denotes g residual reducing sugar. ℓ^{-1} after 76 days of fermentation.

In the presence of solids but without nitrogen addition (S_2N_1) TFT was drastically reduced to the same level (viz. 26,5 days) for both WE 452 and WE 500, while for WE 14 and WE 432 TFT was virtually unaffected (residual reducing sugar concentration being, however, once again lower). In the case of nitrogen addition in the presence of solids (S_2N_2) TFT was reduced for all four strains to 16,0, 19,0, 59,3 and 40,0 days for WE 452, WE 500, WE 14 and WE 432 respectively.

From the above it is evident that the relationship between the presence of solids and the addition of nitrogen and its effect on TFT holds true in general. When, however, yeast strains WE 432 and WE 14, were used, the addition of nitrogen in the absence of solids did not decrease TFT. Similarly the presence of solids at too low a level of nitrogen did not decrease TFT for these two yeast strains (Fig. 2A). The yeast strain WE 500 reacted somewhat differently and seemed to be much more sensitive to solids than to the addition of nitrogen. WE 452, on the other hand, completed fermentation in the absence of both solids and additional nitrogen, while these two factors individually and jointly decreased TFT.

As far as quality is concerned, it was previously established in this paper that the addition of nitrogen increased, while the presence of solids decreased wine quality unless, in the latter instance, nitrogen was added (Table 3). This seems to be true when evaluating the effects of these treatments on the yeast strains individually (Fig. 2B). WE 452, however, seems to be more sensitive to the lower nitrogen level at which poorer wines were yielded.

It is evident from Fig. 2 that to shorten TFT without loss of quality the S_2N_2 treatment for all yeast strains as well as the S_1N_2 treatment for WE 452 merits further study. The effect of these treatments on TFT and wine quality is given for the different fermentation temperatures in Table 4.

Although the data in Table 4 do not always differ significantly from column to column it is evident that there is a tendency for quality to be higher in the case of WE 452 when lower fermentation temperatures are applied. For WE 14 and WE 500 it is just the opposite, 15° C favouring the better quality wines. When WE 452 is used as yeast strain it is clear that must should be as clear from solids as possible, while fermentation should proceed at 10° - 13° C. Under these conditions TFT's of 25-39 days will be encountered without quality being adversely affected. However when WE 500 is used, traces of solids must be present and fermentation conducted at 13° C -15° C causing TFT's of 12-18 days, once again without effect on wine quality. Yeast strains WE 14 and WE 432 should not be used when problems with stuck or lagging fermentations can be expected.

CONCLUSIONS

To regulate fermentation speed and subsequent wine quality the importance of the interaction between the presence of solids, nitrogen level of the must, fermentation temperature and yeast strain employed is evident. In all instances it was found that the nitrogen level of musts should be adjusted (Vos, Crous & Swart, 1980), which has the additional advantage of curbing H_2S production (Vos & Gray, 1979).

Of the four yeast strains employed WE 452 and WE 500 were the only two by which fast fermentation could be obtained while retaining wine quality. In the case of WE 452 no grape solids should be present, while fermentation temperature should be between 10-13° C. At these conditions fermentation will take about 30 days. When WE 500 is used traces of solids must be present to increase fermentation speed, while fermentation temperatures of 13° C-15° C favour higher wine quality. Such fermentations will proceed for, on average, 12-18 days.

Strain WE 500 formed less volatile acidity and produced wine requiring less SO_2 than WE 452. Under practical conditions, where some solids are usually present, strain WE 500 causes fermentation times as short as does WE 452 but with the retention of wine quality. To curb the occurrence of stuck fermentations the yeast strain WE 500 should therefore be used, traces of solids should be present in must, while the level of nitrogen should be adjusted and fermentation, should proceed at 13° C-15° C. At the same time wine quality will not be adversely affected.

It is evident that future research should be aimed at the selection of new yeast strains as well as at the determination of those components of grape solids which so drastically influence both fermentation speed and wine quality.

TABLE 4
The effect of yeast strain, grape solids and fermentation temperature on total fermentation time (TFT) and wine quality (nitrogen was added to a level of 520 $mg.\ell^{-1}$)

Yeast strain	Grape solids	Temperature					
		10° C		13° C		15° C	
		TFT (days)	Qual. (%)	TFT (days)	Qual. (%)	TFT (days)	Qual. (%)
WE 452	None	39	62,1	25	59,3	18	53,7
WE 452	Traces	21	54,2	15	50,8	12	48,2
WE 500	Traces	27	51,4	18	55,1	12	57,9
WE 14	Traces	76	51,4	61	53,7	41	63,0
WE 432	Traces	61	49,1	41	47,0	18	50,9

LSD (TFT) $P \leq 0,05 = 5,9$ days
$P \leq 0,01 = 6,8$ days
LSD (Quality) $P \leq 0,05 = 10,0\%$
$P \leq 0,01 = 11,9\%$

Calculated for the yeast strain x nitrogen x solids x temperature interaction.

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