

# Nutritional Deficiencies of Clarified White Grape Juices and Their Correction in Relation to Fermentation

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Submitted for publication: January 1986

Accepted for publication: April 1986

Keywords: grape must, retarded fermentation, yeast nutrition, ergosterol, fatty acids, nitrogen.

**Juice filtrates of grape samples of the cultivars Cape Riesling, Chenin blanc and Colombar from different wine regions of South Africa, were fermented under standardised anaerobic conditions at a temperature of 15°C. Juice clarification reduced fermentation rates but marked differences between the cultivars were observed. Whereas most of the Cape Riesling filtrates fermented dry at a moderate rate, fermentation of the Colombar juice filtrates were strongly retarded or lagged. The Chenin blanc juices fermented at a rate intermediate to these two. It was established that all slow fermentations were caused by nutritional deficiencies of the juices as a result of filtration and that fermentation could be restored to normal by addition of combinations of ergosterol and unsaturated fatty acids.**

The fermentation rate of grape must and hence also the fermentation time are not only important in the formation of the fermentation bouquet and consequently wine quality, but also have major economic implications as they affect the production capacity, and thus the financial return, of wine cellars. Effective management can also be more readily achieved by the possibility of accurately predicting the duration of fermentation.

During the sixties formulas were developed to predict fermentation rate using variables such as sugar, acid contents, pH, temperature, nitrogen, and biotin concentrations (Ough & Amerine, 1961; Ough, 1964; Ough, 1966a, 1966b; Ough & Amerine, 1966; Ough & Kunkee, 1967, 1968). These formulas were empiric and did not include important variables such as juice turbidity and oxygen concentration. Consequently, in practice, the use of these formulas failed to forecast fermentation rates accurately. It is well-known that fermentations can be completed in a very short time by the use of incompletely clarified juice, by aeration or by the implementation of high fermentation temperatures. However, such conditions are in conflict with established principles for the production of quality white wine where free-run juice, effective juice clarification, exclusion of air and low fermentation temperatures are required for the production of the fruity wine types (Wucherpfennig & Bretthauer, 1970; Ribéreau-Gayon, Lafon-Lafourcade & Bertrand, 1975; Singleton *et al.*, 1975; Bertrand, Marly-Brugerolle & Sarre, 1978; Ribéreau-Gayon, Sudraud & Lafon-Lafourcade 1978; Wenzel & Dittrich, 1978; Williams, Ough & Berg, 1978; Buteau, Duitschaever & Ashton, 1979; Castino, Ubigli & Di Stefano, 1980; Houtman, Marais & Du Plessis, 1980a; Bertrand & Miele, 1984; Tromp, 1984). However, if these fermentation conditions are too stringently applied, especially with regard to juice clarification, fermentation will be retarded or may even lag (Wucherpfennig & Bretthauer, 1970; Ribéreau-Gayon, Lafon-Lafourcade & Bertrand, 1975; Haubs, 1976; Groat & Ough, 1978; Lafon-Lafourcade & Ribéreau-Gayon, 1979; Houtman, Marais & Du Plessis, 1980a,

1980b; Houtman & Du Plessis, 1981; Lafon-Lafourcade, Geneix & Ribéreau-Gayon, 1984; Tromp, 1984). In this respect Houtman & Du Plessis (1986) found that fermentations of juice filtrates of the grape cultivars Colombar, Chenin blanc, Sémillon, Cape Riesling and Muscat d'Alexandrie under strictly anaerobic conditions at 15°C required an average of 60 days. In the case of the first three cultivars fermentations often lagged and sugar residues of from 10 to 50 g.l<sup>-1</sup> were found (Houtman & Du Plessis, 1981, 1986.) These authors found that Cape Riesling and Muscat d'Alexandrie juices which were clarified by no. 3 filters, fermented relatively slowly but most fermentations were completed. However, after clarification by EK-filtration the same juices frequently gave rise to lagging fermentation. Evidently the heterogeneous material causing turbidity in grape juice contains a finely dispersed fraction which is essential for active yeast proliferation and fermentation. A very small amount of this fraction was effective in bringing about good fermentation as shown by high fermentation rates in juice filtrates to which 10% or 20% of settled juice had been added (Houtman *et al.*, 1980a; Houtman & Du Plessis, 1981). The degree of dispersion and the filterability of this yeast growth-promoting turbidity fraction differed from juice to juice (Houtman & Du Plessis, 1981, 1986).

There are many indications that these essential yeast nutrition substances occur in the finely emulsified oily fraction of the juice turbidity (Bertrand & Miele, 1984; Houtman & Du Plessis, 1986). Anaerobiosis is only possible during a limited period if no unsaturated lipids are present in the medium. A great variety of fatty compounds may serve in this respect for instance unsaturated fatty acids, phospholipids, sterols, carotenoids and squalene. In all these cases the unsaturation in the molecule restores the yeast proliferation ability. Under aerobic conditions yeast can dehydrogenate fatty acids to unsaturated fatty acids, which can, under anaerobic conditions, serve as hydrogen acceptors in its metabolism (Nordheim, 1965; Linnane & Kellermann, 1971). Similar observations were made with regard to unsaturated steroids such as ergosterol, lanosterol and

*The authors thank Mr H. Haarhoff for his capable technical assistance.*

episterol (David & Kirsop, 1973; Haukeli & Lie, 1973; David, 1974).

The ergosterol content of yeast cells proliferating under anaerobic conditions is rapidly depleted to a minimum of 1 mg.g<sup>-1</sup> (dry mass) at which level it is incapable of further growth. Such yeasts are therefore unsuitable as an inoculum. In contact with air yeasts produce ergosterol very rapidly, viz. up to 5 mg.g<sup>-1</sup> in 20 minutes and thereafter more slowly to a maximum of 10 mg.g<sup>-1</sup>. Yeast cells are then in an optimal condition to function as an inoculum (Aries & Kirsop, 1977, 1978; Kirsop, 1978; Strydom, Kirschbaum & Tromp, 1982).

Brechot *et al.*, (1966, 1971a, 1971b) found that extracts of the wax layer of grape skins contains oleanolic acid which is able to stimulate yeast growth and fermentation rate in a synthetic medium as well as in grape juice under anaerobic conditions. Additions of ergosterol with Tween 80 detergent had a similar result. Sapis Domercq & Peynaud (1973) accelerated anaerobic fermentation by heat treatment of the grape mash before juice separation, and ascribed this effect to steroids and unsaturated fatty acids from the grape solids. Ergosterol, added to grape juice in a finely dispersed (micro-crystalline) form, had a considerable effect on yeast growth and fermentation rate provided that it was added at the time of inoculation (Houtman, *et al.*, 1980a). Additions of ergosterol/Tween 80 combinations were more effective, even when added to a slowly fermenting must at a later stage (Houtman & Du Plessis, 1981). Investigations with excessive additions of 25 to 100 mg.l<sup>-1</sup> were less effective than additions of 20% or even 10% by volume of settled juice to the filtrates. Lagging fermentations could be corrected even on the 45th day by ergosterol/Tween 80 mixtures, and fermented dry (Houtman, *et al.*, 1980a; Houtman & Du Plessis, 1981). It is understandable that insoluble lipids will only be assimilable by yeasts in a finely emulsified form, and indeed it is known that fermentation stimulating agents must be very finely dispersed.

Recent studies do not ascribe the prevention of lagging fermentation by steroids to stimulated yeast proliferation but to an extension of the active life phase of the yeast cells. These steroids were therefore designated as "survival factors" (Lafon-Lafourcade *et al.*, 1977; Larue, Lafon-Lafourcade & Ribéreau-Gayon, 1978, 1979; Lafon-Lafourcade & Ribéreau-Gayon, 1979; Lafon-Lafourcade, 1980). The role of steroids as "survival factors" for yeast in the final phase of fermentation was confirmed by Traverso-Rueda & Kunkee (1982). Because fermentation rate in such cases is increased from the beginning of fermentation, it can only be explained as a yeast growth and fermentation enhancing effect (Houtman *et al.*, 1980a; Houtman & Du Plessis, 1981).

The key position of oxygen in the production of unsaturated lipids has already been mentioned. During yeast growth the first few generations of yeast can still be produced without oxygen using the ergosterol and unsaturated fatty acid supply of the inoculum. Consequently, air contact during the first 24 hours after inoculation has very little effect on fermentation rate, but fermentation is greatly enhanced by an aeration of the head space on the second or third day after inoculation when the number of cells has already increased consid-

erably and their supply of ergosterol and unsaturated fatty acids have been exhausted (Lafon-Lafourcade, 1980; Soufleros & Bertrand, 1980; Houtman & Du Plessis, 1986). Such an aeration is detrimental to wine quality as the yeast, thus stimulated in the beginning of the exponential fermentation phase, induces a sharp drop in the redox potential and a resulting formation of H<sub>2</sub>S-like odours when the oxygen supply is suddenly withdrawn (Houtman & Du Plessis, 1981).

A factor which can also affect fermentation rate is the yeast strain. There are great differences in the fermentation rates which different yeast strains can maintain as well as in their ergosterol requirements and ability to complete fermentation. Other specific differences among yeast strains appear in the length of lag time after inoculation (Tromp, 1984; Houtman & Du Plessis, 1986). The amount of the inoculum, which seems to be independent of the yeast strain also affects both the lag time and overall rate of fermentation. In the case of small inoculums (0.8 x 10<sup>9</sup> live cells.l<sup>-1</sup>) more yeast cells have to be produced than is the case with large inoculums. The lag time before which fermentation is perceivable is therefore longer. In settled juices yeast proliferation is sufficiently supported by the medium and fermentation curves are parallel and congruent, irrespective of the inoculation concentration (Lewis & Enevoldsen, 1973; Houtman & Du Plessis, 1981). On the other hand, in over clarified juices fermentation depends largely on the amount of unsaturated fatty acids and steroids in the inoculum. With a small inoculum these substances are soon exhausted and yeast proliferation becomes inadequate, causing the fermentation rate to drop considerably before fermentation is completed. With such juices far better results are obtained with large inoculums of up to ca 2 x 10<sup>10</sup> live cells per litre, enriched with ergosterol by aeration shortly before use (Houtman & Du Plessis, 1981).

The possibility of turbidity-producing particles stimulating fermentation mechanically by acting as "carriers" of yeast cells and thus keeping the yeast "floating" and more evenly distributed in the must, was investigated by the addition of artificial turbidity-producing materials such as bentonite, diatomaceous earth, talc or polyamide powder to the must (Ribéreau-Gayon *et al.*, 1975; Haubs, 1976; Groat & Ough, 1978; Tromp 1984). However, these authors differed in opinion on the effect of these substances on fermentation rate and it is more generally accepted at present that lagging fermentation in clarified juices should be attributed partially to nutritional deficiencies of the medium.

Critical nutritive substances can also be lost from the juice by clarification with bentonite, which may cause lagging fermentation. It is therefore recommended, that bentonite, added to the juice for protein stabilisation, should remain in the must during fermentation (Petro, 1965; Ponomarchenko & Albot, 1976; Bach & Hoffmann, 1979; Tsakov *et al.*, 1979).

A shortage of assimilable nitrogen is frequently mentioned as a cause of retarded fermentation (Bizeau, 1963; Lewis & Enevoldsen, 1973; Wenzel & Dittrich, 1978; Vos & Gray, 1979; Tromp, 1984). Bizeau (1963) also noted the possibility of phosphorus and mineral deficiencies. Retarded fermentations have also been ascribed to vitamin deficiencies, i.a. thiamine, pantothen-

ic acid and biotin, but then mostly in combination with other factors (Ough & Kunkee, 1968; Ribéreau-Gayon *et al.*, 1975; Lafon-Lafourcade & Ribéreau-Gayon, 1979; Larue, Lafon-Lafourcade & Ribéreau-Gayon, 1979; Ribéreau-Gayon *et al.*, 1979; Minàrik, 1983). There are, however, no indications that vitamin deficiencies often occur in grape must.

Antibiotics excreted by *Botrytis cinerea* and pesticide residues are mentioned as causes of retarded fermentation (Ribéreau-Gayon *et al.*, 1979; Lafon-Lafourcade, 1980). On the other hand, Minàrik (1983) obtained a yeast growth promoting extract from *B. cinerea*. It would appear that poisoning of the yeast by such external influences is sporadic and does not cause any serious problems.

Like ethanol, octanoic acid and decanoic acid are fermentation retardants which are produced during the fermentation process (Nordström, 1964; Geneix, Lafon-Lafourcade & Ribéreau-Gayon, 1983; Lafon-Lafourcade, 1984; Larue *et al.*, 1984). These fermentation retarding fatty acids can be absorbed from extracted yeast cell-wall material added to the must thus enhancing the rate of fermentation (Geneix *et al.*, 1983; Lafon-Lafourcade, 1984; Lafon-Lafourcade, Geneix & Ribéreau-Gayon, 1984; Larue *et al.*, 1984). However, the effect of added cell-wall material, can more convincingly be attributed to the fact that it also serves as a source of essential yeast nutrients (Bertrand & Miele, 1984).

It is clear that radically clarified grape juice often is an inadequate medium if smooth and complete fermentation, as well as good wine quality, is to be achieved. However, cellars can generally obtain satisfactory results if juice clarification and anaerobiosis are not too drastic, selected yeasts with low nutritive requirements are used and mass inoculation is applied.

Apart from these aspects differences between the fermentability of juice samples on a cultivar basis have been observed (Houtman & Du Plessis, 1986). Cape Riesling and Muscat d'Alexandrie juice filtrates were more suited as a medium for yeast proliferation than Chenin blanc and Colombar. Apparently the fermentation promoting turbidity fraction in the former two cultivar samples was more finely dispersed and filterable than in the latter two (Houtman & Du Plessis, 1986). It is important that the nature of components contributing to effective fermentation of grape juices be identified. The aim of this study was to gain insight into the nature of compounds involved in fermentation differences between specific South African grape cultivars.

## MATERIALS AND METHODS

### Juice preparation and fermentation:

Wines were made from Chenin blanc, Cape Riesling and Colombar grapes from the Stellenbosch, Lyndoch, Paardeberg, Paarl, Huguenot, Robertson and Lutzville areas.

Free run juice was collected and settled overnight at approximately 3°C. The sulphur dioxide content was corrected to ca 75 mg.l<sup>-1</sup> prior to inoculation. Juice was filtered after settling through no. 3 filter sheets which was followed by an EK-filtration. All juice was saturated with carbon dioxide and all subsequent manipulations carried out anaerobically in a closed system under this gas. Juice samples were all held at -25°C in 5

litre glass containers until required.

An inoculation of 0,2 g.l<sup>-1</sup> dry yeast (*Saccharomyces cerevisiae*; WE 14 of VORI collection) was used. Living cell counts were ca 8,8 x 10<sup>8</sup> cells.l<sup>-1</sup> per inoculation. Standardised anaerobic fermentations were carried out in five-fold with 0,4 litre juice samples (Houtman & Du Plessis, 1981, 1986) at 15°C.

### Treatments:

All juices received 0,4 g.l<sup>-1</sup> di-ammonium phosphate, 0,45 mg.l<sup>-1</sup> thiamine and 0,07 mg.l<sup>-1</sup> biotin. Twenty percent (v/v) solutions were made of yeast extract, malt extract and peptone and briefly heated to 100°C. Tomato juice was clarified by filtration and used as such. Additions of these water soluble nutrients were given in volumes which, either in combinations or individually contained, per addition approximately 100 mg.l<sup>-1</sup> nitrogen (expressed as NH<sub>3</sub>) as determined from their specified nitrogen contents. Since the effects of individual or combined additions were practically similar their results were consequently combined and their mean values used.

The unsaturated fatty acids oleic, linoleic and linolenic (60-300 mg.l<sup>-1</sup>) were added individually or in combinations with or without ergosterol (15-25 mg.l<sup>-1</sup>).

The ergosterol (finely powdered) was dissolved in the slightly warmed mixtures of the fatty acids. All mixtures were finally diluted with 0,1 ml ethanol and saponified in 20 ml 0,3 % (m/v) potassium hydroxide. The results of the different fatty acid additions showed little difference and were consequently combined.

### Fermentation rates and times:

Carbon dioxide mass loss was determined over time. Fermentation times were determined over two periods viz. the time required for sugar consumption from 5-50 percent (Ft 5-50) and the fermentation time from 0-99 percent of sugar consumption (Ft 0-99) (Houtman & Du Plessis, 1986). In cases of lagging fermentation the 99 percent point was determined by extrapolation.

In this study fermentations between 0-99 percent sugar utilisation which were regarded as non-retarded, retarded or lagging between sugar concentrations of 150-240 g.l<sup>-1</sup> in the juice, were empirically defined as given below.

1. Juice fermentations were regarded as non-retarded if the times taken to ferment were less than the values given by the following formula:

$$\text{time limit} = 25 + \frac{(\text{juice sugar in g.l}^{-1} - 150)}{3} \quad \text{days}$$

For 150 to 240 g.l<sup>-1</sup> sugar these values are 25 to 55 days.

2. Juice fermentations were regarded as lagging if the time taken to ferment exceeded the values given by the following formula:

$$\text{time limit} = 55 + \frac{(\text{juice sugar in g.l}^{-1} - 150)}{3} \quad \text{days}$$

For 150 to 240 g.l<sup>-1</sup> sugar these values are 55 to 85 days.

3. Juice fermentations were regarded as retarded if the times taken to ferment juice with sugar concentrations between 150 and 240 g.l<sup>-1</sup> were between the values given for non-retarded and lagging fermentations under 1 and 2.

## RESULTS AND DISCUSSION

During filtration of grape juice through no. 3 filter it was found that due to partial blockage of the filter by

grape particles, it later passed only exceptionally small particles (Houtman & Du Plessis, 1981). These filtrates were such that their fermentation characteristics closely resembled those of EK-filtrates. As a result of this phenomenon the data from no. 3 and EK-filtered juices were combined.

#### Differences in fermentability of juice filtrates of three grape cultivars:

Marked differences were found between the juices of the cultivars Cape Riesling, Chenin blanc and Colom-

TABLE 1

Analytical data and fermentation times (Ft) of juice filtrates from three grape cultivars.

Component/Period	Cape Riesling		Chenin blanc		Colombar	
	Analysis					
	Mean value	Cv	Mean value	Cv	Mean value	Cv
Sugar (g.l <sup>-1</sup> )	190	8	192	10	196	11
Total acid (g.l <sup>-1</sup> )	4,4	15	6,4	16	7,1	15
pH	3,59	5	3,37	5	3,31	6
SO <sub>2</sub> : Free (mg.l <sup>-1</sup> )	25	24	35	31	47	19
Total (mg.l <sup>-1</sup> )	48	31	65	28	80	29
	Fermentation times (days)					
	Ft	Cv	Ft	Cv	Ft	Cv
5 to 50% (Ft 5-50)	5,1	18	6,2	24	6,6	17
0 to 99% (Ft 0-99)	38	32	48	23	53	19

Cv: Coefficient of variation (%)

TABLE 2

Classification of juices according to cultivar and fermentation rate

Fermentation rate class	Number of juice samples per cultivar		
	Cape Riesling	Chenin blanc	Colombar
Non-retarded	9	3	1
Retarded	2	11	3
Lagging	1	4	6

bar with regard to analytical data and fermentation times (Table 1). Total acid and free and total sulphur dioxide concentrations in Colombar juices were always considerably higher and pH lower than in the Cape Riesling juices. The SO<sub>2</sub> content of the Colombar juices was close to the added concentration of 75 mg.l<sup>-1</sup> whereas in the Cape Riesling juices roughly half of this value was found (Table 1). Obviously the grape solids in the Cape Riesling mash have a rather strong SO<sub>2</sub>-binding capacity. However, these differences between Colombar and Cape Riesling, in themselves, give no sufficient explanation for the higher fermentation rates of the Cape Riesling juices. None of these tabulated data are critically high or low with respect to fermentation retardation and these fermentations were not impaired or stimulated by varying of their pH or SO<sub>2</sub> concentrations within these ranges (A.C. Houtman, unpublished data). Nevertheless, these pH and SO<sub>2</sub> differences could be additional retarding factors in juices with nutritional deficiencies.

The forty juices and their fermentation times up to 99% sugar consumption were distributed over three fermentation rate classes viz. non-retarded, retarded and lagging (Table 2). These results confirm the observations on a smaller scale with the same grape cultivars over the last five years (A.C. Houtman, unpublished data). The most obvious differences are those between Cape Riesling and Colombar juices where the former fermented at a faster rate than the latter. Chenin blanc generally fermented at a rate between that of Cape Riesling and Colombar (Table 2).

#### Water-soluble nutrients and unsaturated lipids:

Table 3 shows that retardation of fermentation in the thoroughly clarified juices could be attributed entirely to nutritional deficiencies. For example the mean fermentation times of the Colombar juices could be reduced from 64 days to 23 days with a combined addition of water-soluble nutrients and unsaturated fatty acids and ergosterol. The most active components in this respect were ergosterol and unsaturated fatty acids.

TABLE 3

Effect of water-soluble nutrients, unsaturated fatty acids and ergosterol on fermentation time (Ft 0-99%) of de-aerated juice filtrates of three grape cultivars\*

Juice addition				Fermentation times (days)					
Water-soluble nutrients (mg. NH <sub>3</sub> .l <sup>-1</sup> )		Unsaturated fatty acids		Cape Riesling		Chenin blanc		Colombar	
A	B	C	Ergosterol (mg.l <sup>-1</sup> )	Ft	Cv	Ft	Cv	Ft	Cv
—	—	—	—	32	22	46	30	64	50
100	—	—	—	34	7	43	21	54	9
—	100	—	—	34	10	44	9	56	15
—	—	80	—	28	11	35	16	47	18
—	—	80	6.7	24	5	29	21	32	12
—	100	80	6.7	21	3	28	17	23	39

\*6 Cape Riesling, 13 Chenin blanc and 8 Colombar juices.

A: A mixture of 0.4 g di-ammonium phosphate, 0.45 mg thiamine and 0.07 mg biotin.l<sup>-1</sup>.

B: Yeast extract, peptone, malt extract and clarified tomato juice, individually and in combination had about the same effect and results were consequently combined.

C: Oleic, linoleic and linolenic acids individually and in combination, had about the same fermentation promoting effect and results were therefore combined.

Cv: Coefficient of variation (%)

TABLE 4

The effect of additions of yeast nutrients to juices in three classes of fermentation time (Ft 0-99%) viz. non-retarded, retarded and lagging fermentations\*

Juice additions				Fermentation times (days)					
Water-soluble nutrients (mg. $\text{NH}_3\text{.l}^{-1}$ )		Unsaturated fatty acids		Non-retarded		Retarded		Lagging	
A	B	C (mg. $\text{l}^{-1}$ )	Ergosterol (mg. $\text{l}^{-1}$ )	Ft	Cv	Ft	Cv	Ft	Cv
—	—	—	—	35	17	49	10	85	29
100	—	—	—	36	12	40	11	67	7
—	100	—	—	36	9	44	12	74	16
—	—	80	—	29	13	37	16	58	22
—	—	80	6,7	26	11	27	13	42	12
—	100	80	6,7	24	6	20	23	27	39

\*The number of juices in the three groups were 17, 8 and 7 respectively and the juices were selected from all three cultivars.

A: A mixture of 0,4 g di-ammonium phosphate, 0,45 mg thiamine and 0,07 mg biotin. $\text{l}^{-1}$ .

B: Yeast extract, peptone, malt extract and clarified tomato juice, individually and in combination had about the same effect and results were consequently combined.

C: Oleic, linoleic and linolenic acids individually and in combination, had about the same fermentation promoting effect and results were therefore combined.

Cv: Coefficient of variation (%)

Only in the case of Colombar could the fermentation supporting qualities of juices be improved by additions of di-ammonium phosphate and preparations rich in assimilable nitrogen compounds, such as yeast extract, peptone and tomato juice which also contained fatty acids but their effect with respect to fermentation appears minimal (Table 3).

The combined nitrogen/fatty acid fermentation supporting effect, occurs regardless of cultivar. In Table 4 it is shown that the effect of these additions is most pronounced in those juices where the most serious lagging occurs, indicating that they were indeed lacking specifically in these unsaturated lipids and to a lesser degree in assimilable nitrogen.

Table 5 shows that these juice deficiencies were not affected by grape maturity. They were eliminated at both high and low sugar concentrations by a combination of assimilable nitrogen, unsaturated fatty acids and ergosterol.

#### Unsaturated lipid requirements of yeasts:

In Table 6 it is shown,

1. that 22 mg. $\text{l}^{-1}$  of unsaturated fatty acids appeared to be relatively close to an optimal required concentration since a further increase did not cause a clear corresponding decrease in Ft 5-50 or Ft 0-99;
2. that the effect of ergosterol in combination with unsaturated fatty acids and, provided that it is supplied

TABLE 5

Effect of additions of yeast nutrient on the fermentation time (Ft 0-99%) of low and high sugar content juice filtrates\*

Juice additions				Fermentation time (days)			
Water-soluble nutrients (mg. $\text{NH}_3\text{.l}^{-1}$ )		Unsaturated fatty acids		"Low sugar" juices (160-194 g. $\text{l}^{-1}$ )		"High sugar" juices (195-225 g. $\text{l}^{-1}$ )	
A	B	C (mg. $\text{l}^{-1}$ )	Ergosterol (mg. $\text{l}^{-1}$ )	Ft	Cv	Ft	Cv
—	—	—	—	43	50	55	43
100	—	—	—	34	12	54	15
—	100	—	—	42	13	51	11
—	—	80	—	35	16	41	16
—	—	80	6,7	30	18	30	18
—	100	80	6,7	24	30	25	40

\*Seventeen juice samples in the "low sugar" group and 15 in the "high sugar" group.

A: A mixture of 0,4 g di-ammonium phosphate, 0,45 mg thiamine and 0,07 mg biotin. $\text{l}^{-1}$ .

B: Yeast extract, peptone, malt extract and clarified tomato juice, individually and in combination had about the same effect and results were consequently combined.

C: Oleic, linoleic and linolenic acids, individually and in combination, had about the same fermentation promoting effect and results were therefore combined.

Cv: Coefficient of variation (%)

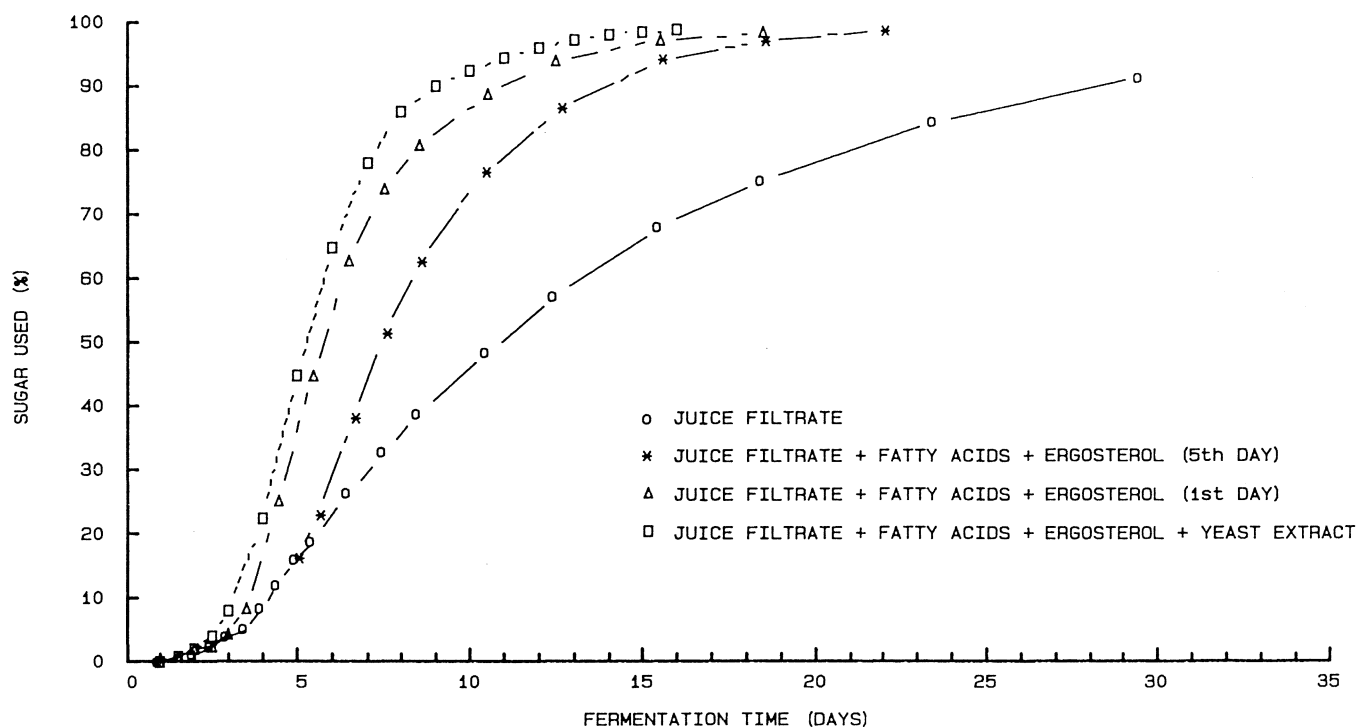


FIG. 1

Fermentation curves of juice filtrates with additions of yeast extract, unsaturated fatty acids and ergosterol at inoculation and on the fifth day thereafter. The percentage sugars used was derived from total  $\text{CO}_2$  mass loss, reducing sugar remaining and  $\text{CO}_2$  mass losses in the interim period.

in a finely dispersed and absorbable form, is already pronounced at a level of 1,7 mg. $l^{-1}$ . The concentration range used here viz. from 1,7 to 6,7 mg. $l^{-1}$  is approximately in line with the nutritional requirements for the proliferating yeast as determined by Strydom *et al.*, (1982).

#### Correction of retarded fermentation by addition of unsaturated lipids:

In Fig. 1 it is shown that fermentation rate can be stimulated on the fifth day after inoculation by an ad-

dition of the unsaturated fatty acids plus ergosterol preparation. This effect was nearly equivalent to that of the same additions at the time of inoculation. A marked improvement in fermentation rate was brought about by the unsaturated lipids even at a relatively late stage of fermentation. This effect could not be achieved by the sole addition of large amounts of dispersed and absorbable ergosterol, under similar conditions (Houtman & Du Plessis, 1981).

#### CONCLUSION

It was evident from this study that clarification of juice can remove substances which are essential for yeast proliferation. This effect can radically decrease fermentation rate but can differ between cultivars. Since a rapid, sound fermentation is important to wine makers, it appears necessary that the effect of normal cellar practices of juice clarification on the removal of fermentation promoting substances be investigated.

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TABLE 6  
The effect of three concentration levels of unsaturated fatty acids and ergosterol on the fermentation time (Ft) of grape juice filtrates\*

Additions		Fermentation time (days)			
Unsaturated** fatty acids (mg. $l^{-1}$ )	Ergosterol (mg. $l^{-1}$ )	Ft 5-50	Cv	Ft 0-99	Cv
0	0	7,1	5,0	50	0
20	0	4,9	0,7	42	1,7
40	0	4,9	2,7	42	2,4
80	0	4,6	7,2	38	8,7
20	1,7	4,1	1,7	35	0
40	3,3	3,5	3,1	30	0
80	6,7	2,9	1,0	25	6,8

\*Two parallel fermentation series with Chenin blanc and Colombar juices, enriched with di-ammonium phosphate at 0,4 mg. $l^{-1}$ .

\*\*A mixture of 20% oleic acid, 40% linoleic acid and 40% linolenic acids.

Cv: Coefficient of variation (%).

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