RECENT ADVANCES IN THE PROCESS OF FLOTATION APPLIED TO THE CLARIFICATION OF GRAPE MUSTS

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Abstract

Flotation applied to grape must and fruit juice clarification has found, during the past few years, a greate applicatory interest.
The system allows the continuous treatment of a large quantity of raw must. It is possible to guide the liquid-solid separation up to the degree of limpidity desired, rationalizing the use of fining agents.
This process has been used together with the techniques of hyperoxygenation and cross-flow filtration of must; therefore this separation technique can be considered innovative both in regard to the process of elaborating stable wines and the production of new products, for example wines without sulphur dioxide.

Introduction

Evolution in the technology of grape must and fruit juice clarification has found, during the past few years, in the technique of flotation, a process of greate applicatory interest. It involves the separation of the liquid from the naturally lighter solids, or those made so, by their adhesion to micro-bubbles of gas.
In the field of oenology, following the initial tests using pilot plants (WAJSFELNER, 1989; OTTO AND STROHM, 1987; FERRARINI, 1990; FERRARINI *et al.*, 1991*a*; BARDINI AND MAGGI, 1992), which did not always lead to satisfactory results on an industrial scale, there was a spread of the continuous system with the dissolution of gas (air or nitrogen) by means of direct pressurisation at 5 bars in the raw must, using a circular flotation tank with reduced flaps and a central distribution of the flow (FERRARINI *et al.*, 1992*b*).
The system constructed in such a way allows the continuous treatment of a large quantity of must, the process is thus often an operation that is fundamental, even from a qualitative aspect, in the processing of white and rosé wines.
In addition flotation has been used together with the techniques of hyperoxygenation and cross-flow filtration of musts (ZIRONI *et al.*, 1993; FERRARINI *et al.*, 1992*a;* FERRARINI *et al.*, 1991*b;* TOURNIER, 1990; TROUSSEAU AND CHAPRON, 1991).
Therefore this separation technique can be considered innovative both in regard to the process of elaborating stable wines and the production of new products (for example wines without sulphur dioxide) (ZIRONI *et al.*, 1993).
Considering the complexity of the aspects involved in this sphere, flotation applied to the clarification of musts could be the subject of further investigation both from the engineering and technological point of view.
In the present paper some results of recent experiments on these aspects are presented.

New acquisitions in regard to the engineering achievement of the flotation plants

Previous studies (FERRARINI *et al.*, 1991*b*) and different industrial applications have demonstrated the validity of the system with a circular flotation tank and a central distribution of flow. The latter

could be the source of some perplexity in regard to the homogeneous distribution of the lees of flotation; therefore the distribution of the layer of lees within the flotation tank was evaluated during the procedure. The apparatus, mod. MEX-3 produced by BTG, furnished with an immersion probe using infrared reys, mod. RD-10/5; was calibrated using water for the zero setting and grape must containing 30% w/v solids (determined by centrifugation at 3000 rpm x 5 min) for the highest level of suspendes solids.Thus a calibration curve was plotted capable of evaluating the solid contents of the flotation lees present on the surface in an industrial system of 200 hL/h.
Figure 1 shows a graphic representation of these results, the measurements were made at different depths along the entire lenght of a ray of the flotation tank. A regular distribution of the flotation lees can be observed, which at a shallow depth (5-10 cm) over the surface reaches a value of 40 % w/v solids in suspension. Lower down, to a depth of 20 cm, there is always lees than 1% solids for the entire ray of the tank. This distribution ensures a good rate of recovery, in the lower parts, of a clear must and well compacted sediment on the surface.
A homogeneous distribution of the concentration of the solids inside the flotation tank indicated that there is good drainage from all the areas of the lees layer. In fact, the separation of the liquid within the layer of floating sediment can be compared to filtration; and thus Darcy's law is applicable:

$$\frac{dV}{dt} = K\frac{A\,\Delta p}{\eta\, l}$$

where:

$\frac{dV}{dt}$ = the speed of permeation of the liquid;
K = specific permeability of the filter medium;
A = the filtration surface;
Δp = pressure difference through the filter medium;
η= viscosity of the liquid;
l = thickness of the filter medium.
The law dictates thet the speed of permeation is proportional to the difference in pressure, the area of filtration and inversely proportional to the viscosity and the depth of the filter medium. A parameter to note is the factor K which is linked to the tipology of the must solids and the drainage system given by the fining agents.
Figure 2 shows the results of flotation carried out in the laboratory with cylinders of increasing height (consequently with an increasing ratio of volume/surface) and using must with a 5,5 % content of suspended solids. It can be seen that for the minimum observation time of 30 minutes the speed of separation, with the volume/area ratio considered were such as not to permit significant differences in the separation yield; evidently given the low content of solids, the factor "l" (the layer of lees) of Darcy's law conditions the speed and yield of separation over relatively short periods and of little technological interest. Further confirmation is provided by the yield of sediment observed after 60 minutes: the different ratio of volume/surface area do not give variations in yield while a longer resting period brings about a decrease of about 3% in the quantity of floating lees.
Figure 3 illustrates the results of an experiment on flotation carried out on the same must as used in the previous test but enriched with different percentages of suspended solids.Evaluating the separation effect after 10 and 60 minutes of resting, it can be observed that the speed of separation (dV/dt) is distinctly lowered to such values that after 10 minutes there are significant differences in yield only for quantities of suspended solids equal to, or above, 12%. Evidently the high values of the ratio solids/liquid make the "l" factor of Darcy's law important for a large part of the separation process, so that in this case the speed of flotation is only slightly effected by Stokes law. Thus whenever one works with musts containing high values of suspended solids the situation of separation that is created in the medium becomes more similar to a filtration process characterised by

the movement of the filter medium in the liquid and not vice versa as occurs in a normal system of filtration. The factor Δp of Darcy's law that determines the transfer of material (liquid) through the filter medium is in this case given by the hydrostatic push possessed by the filter medium of "l" thickness. In addition, the same figure indicates that, for the waiting time of 60 minutes, the values of solids even up to 14% do not sensibly reduce the yield of the separation process.
Figure 4 describes the changes in the percentage of flotation sediment during the process of separation of musts with a percentage of suspended solids varying from 4 to 6%: it can be seen that during the first ten minutes there is the separation of the greater part of the lees and with resting times above 30-40 minutes there is no improvement in the yield from the separation process.
Longer times give a further separation of limpid juice according to the reaction shown in Fig. 5. This experiment, which was carried out on flotation lees obtained with an industrial plant, has shown that following a further three hours of resting there is a separation from limpid must of 15-20% of the total quantity of lees; this greater rate of separation can be used to advantage in the discontinuous flotation processes.
These different parameters must be adequately evaluated for the measurements of the flotation systems.

Study of the phenomena connected to the flotation process

The speed of solid-liquid separation occurring in flotation, whether regulated by Stokes's or Darcy's law is, in any case, influenced by viscosity (η) in an inversely proportional manner. Therefore the enzymatic effect on pectin, which is mostly responsible for the viscosity of the medium, can have a favourable effect on the separation process.
Table 1 reports the data of a series of experiments on flotation with or without pectolytic enzymes. The parameters of limpidity and percentage of lees separated were significantly modified, $p = 0.05$ and $p = 0.01$ respectively, due to the enzymatic effect. The enzymatic action can transform pectin into hydrophobic colloids (LE QUERE *et al.*, 1988): these combine better with the gas and accentuate the phenomena of syneresis; in this way together with an improved drainage of the solids there is a production of lees with a lower percentage of liquid.
In reality the enzymes modify the separation process not only in regard to speed and yield, but also quality. In fact the pectin polymers are broken down and the colloidal state destabilised, thus permitting a greater activity of the fining agents and as a consequence a greater limpidity. The improved effect on the separation of solids leads to a slight transfer of some substances that they contain, in particular total nitrogen, catechins and more generally phenolics substances. This fact is confirmed by the analysis of variance which shows a correlation between enzymatic action and phenolic and nitrogen fractions. The hypothesis of a reaction due to the activity of secondary enzymes present in the commercial preparation that could accentuate the oxidative catabolism of the phenolic substances starting from the cinnamic compounds (CHATONNET *et al.*, 1993), should not be underrated. This would explain the lower amount of catechins in the enzymatic tests.
The effect of enzymatic treatment is closely linked to the time of reaction; Fig. 6 shows that the greater part of the reaction due to the added enzyme, calculated as limpidity, occurs during the first three hours of contact. The action of the enzyme on the percentage of floating lees becomes evident, as already noted, by a reduction in its amount, detectable only for enzymatic reaction times of about 30 minutes which correspond, for the type of must being tested, to a turbidity below 100 NTU. Longer times of contact with the enzyme lead to an increase in the percentage of solids separated due to the effect of excessive fragmentation of the pectic substances.
In contrast, Fig. 7 shows the effect on the efficiency of the separation process of the quantity of pectolytic enzymes used before flotation. Under the specific experimental conditions and for a resting time of 60 minutes at 20°C, there is an improvement in the limpidity up to a dose of 4 g/hL. Evaluating the percentage of flotation lees separated, it can be seen from Tab. 1 that there is an improvement in yield due to the use of enzymes, even if the higher amounts increase the percentage of sediment separated for a greater separation of solids from liquid.

The behaviour of different commercial preparations was evaluated; the results given in Tab. 2 show that they are significantly different, both in regard to the clearing and the separation effect.
If the action of the pectolytic enzymes is often an essential preliminary for flotation, then the greatest effects of separation are given by the fining agents.
An essential condition for separation by means of flotation with gas at a differential pressure is the ability of the floccule to bind with the gas, generally air or nitrogen. This is determined by the degree of hydrophobicity of the floccule: if this is high the angle of contact with the gas is increased, thus favouring flotation (WAJSFELNER, 1989).
In the flocculation process the superficial electrostatic charge on the colloids must be neutralised to render them hydrophobic (RIBEREAU-GAYON *et al.*, 1980). Thus it is of fundamental importance to know the value of the superficial charge ("Zed" potential) of the solids dispersed in the must. On an industrial scale notable differences in the behaviour of musts has been observed, in particular the final fraction extracted from the pressed grapes requires abnormal quantities and proportions of clarifying agents.
Using a Streaming Current Detector, mod. PCD 02 produced by Mütek, the superficial charge was measured of the solids from a must obtained from the first and last pressing as shown in Tab. 3. It is interesting to note that, even though the grapes are the same, the charge is different (+6 mV the must from the first pressing, -14 mV the must from the last pressing) which could explain the different behaviour in regard to the clarifying agents (DIETRICH *et al.*, 1992). In particular it is important to remember the role played by the content of phenolic substances for the caracterisation of the electric charges as is confirmed by the data shown in Fig. 8. This experiment carried out on Trebbiano must treated by flotation in the presence of increasing amounts of phenolics substances (addition of tannin) shows how the increase in the content of polyphenols decreases the separation of solids, made evident by the increase in turbidity; in addition it is interesting to note that the lowest amount of turbidity is obtained by adding 20 mg/L tannin, evidently the ratio gelatine/phenolic substances is in this case optimal for adhesion to the flotation gas.
Fig. 9 reports some flotation tests carried out using various fining agents at different proportions and amounts. The high dispersion of the data indicates the absence of a correlation between limpidity and the percent of lees floated. The combinations of fining agents can be determined that are able to gratify both the separation aspect (percent of lees) and that of limpidity.
This behaviour can presumably be explained by the "syneresis" effect which occurs during compacting of the floccules which collapse due to the force of the surface tension between dehydrated and hydrophobic micelles (RIBEREAU-GAYON *et al.*, 1980). This phenomenon is shown in a schematic form in Fig. 10: it represents separation by means of flotation in a cylinder and indicates with the letter C the limpid fraction (71 %) obtained during the first phase of the flotation, A is the leesl (21%), while B is a small fraction (8%) of much clearer must obtained with longer separation times when the phenomenon of syneresis becomes more marked.

Technology of the process

The principal result of flotation is the separating out of the solids present in the must with the well known effects on the quality of the product (GROAT AND OUGH, 1978; HOUTMAN AND DU PLESSIS, 1981; RIBEREAU-GAYON *et al.*, 1980; TROMP, 1983; VAN ROOYEN AND TROMP, 1982). Typically it is used to clarify white and rosé musts, elaborated in the absence of solid substances or with skin contact or at low temperatures; in the elaboration of red wines flotation can be used for the clarification of musts obtained by skin contact at high temperatures.
In addition, in contrast to other separative techniques, some random and not always applicable on an industrial scale, others excessively efficient and at times with a negative effect on the fermentation process (OLLIVIER *et al.*, 1987; DELFINI AND COSTA, 1993), flotation permits the desired level of limpidity to be easily controlled, thus wines of a superior quality are produced (DUBOURDIEU *et al.*, 1986). The separative effect of flotation can be used as a pretreatment in membrane separation processes: inverse osmosis (GUIMBRTEAU *et al.*, 1989) and cross flow filtration (FERRARINI *et al.*, 1991b; ZIRONI *et al.*, 1993).

Flotation is usually carried out using fining agents, as a rule: gelatine, bentonite and silica sol; their continued use permits the time of contact to be enormously reduced thus limiting the transfer of unwanted substances.
The use during flotation of oxidising gases (air or oxygen), can provoke a hyperoxygenation effect on the musts, this technique has been used for some time with often satisfying results on the stability of the wine (MÜLLER-SPÄTH, 1977; AA.VV., 1990; GUERZONI *et al.*, 1981; ARFELLI *et al.*, 1992).
Also in flotation the hyperoxygenation can give positive results on the stability of the wines, as already shown in previous experiments (FERRARINI *et al.*, 1992*a*; ZIRONI *et al.*, 1993). However the information furnished by different investigators is not always in agreement concerning the way to carry out this technological process.
For this reason some tests were carried out to evaluate the different technological parameters that could interfer with the process of hyperoxygenation.
First of all, the quantity of oxygen and the times necessary for the completion of the enzymatic action on the phenolic substances were rechecked. Some experiments were also carried out to measure the absorption of oxygen, at atmospheric pressure and room temperature, by musts of some cultivars; the method used was that suggested by GÉTAZ AND FABRE, 1990.
Some of these results are shown in Fig. 11; it can be noted that the musts of the grapes considered differ from each other in regard to their reactivity with oxygen, evidently this is linked to the amount of enzymes and the composition of acceptors that characterise the substrate (CHEYNIER *et al.*, 1989, 1990, 1991; SINGLETON, 1987). In addition, the curves show that the quantities of oxygen absorbed, even over quite long periods of time, is not more than 30 - 40 mg/L.
The kinetics of hyperoxygenation have been studied evaluating the principal components involved in the reactions caused by oxygen. Fig. 12 reports the results of some hyperoxygenation experiments carried out on grapes of the Garganega cultivar in the presence of a constant concentration of dissolved oxygen (about 80 mg/L). From the graphs it can be seen that the greater part of the modifications induced by hyperoxygenation occurs over a period of 60 minutes. In particular the total phenolic content is reduced by more than 30 % of the initial values; at the same time catechin, on average, is reduced by about 70 %. The kinetics are confirmed by the O.D. 320 nm values which correspond to the maximum of optical absorption of hydroxycinnamiltartaric acids: these too are the object of a strongly active oxydative metabolism in the first 10 - 20 minutes and which is always completed within 60 minutes. It is interesting to note the continuous decrease in colour of the musts (O.D. 420 nm) which together with that due to the fermentation (RIBEREAU-GAYON, 1980; CANTARELLI *et al.*, 1992), leads to a wine with the same colour and more stable those produced using traditional techniques.
In addition experiments were carried out to evaluate the influence of the oxygen concentration on yhe kinetics of the hyperoxygenation reaction; Fig.13 shows the trend of the two most significant parameters: total polyphenols and catechins. These trends show that the kinetics are not, quantitively and qualitatively, influenced in a significant way not even by the concentrations of oxygen present in the medium in quantities greatly exceeding in respect to that required at atmospheric pressure (5 bars oxygen pressure corresponding to more than 200 ppm of dissolved gas); which confirms the prevalently enzymatic process of the oxidation of musts.
Instead Fig. 14 reports the course of total polyphenols and catechins in similar test on hyperoxygenation carried out at different temperatures (7, 20 and 35 °C): the graphs indicate that the reaction kinetics are clearly influenced by temperature. In particular the decrease with time of total polyphenols and catechins is slowed both by low (7°C) and high temperatures (35 °C). This can be easily explained at low temperatures to reduce the oxidative activity of the enzymes, while the high temperatures, though considerably increasing the speed of these reactions, provoke a dissolution of the solids, still present in the medium, and of phenolic substances at a higher quantity than that subjected to oxidative condensation.
Finally Fig. 15 shows results of experiments on hyperoxygenation carried out both with and without the addition of fining agents. The kinetic progress indicates that the presence of fining agents (gelatine, bentonite, silica sol) do not hinder the oxydative action of the endogenous enzymes, indeed

apart from the phenolic substances they are rapidly absorbed by the fining agents, thus improving the stabalising action of the hyperoxygenation.
Therefore on the bases of the results of these recent investigations and what was already known (AA.VV., 1992), the most favourable conditions can be determined for improving the stability of wines using the technique of hyperoxygenation of the musts during the course of flotation, which in this case will be engineered on the bases of determined parameters.
As an example in Tab. 4 some determinations carried out on grape musts of the cultivar Picpoul clarified using the traditional techniques and by means of flotation, the latter with or without sulphur dioxide, and thus under conditions respectively of hyperoxygenation or not (ZIRONI *et al.*, 1993).
The analytical results indicate that due to the action of the oxygen in the absence of sulphur dioxide, there is a notable lowering in the amount of phenolic substances, in particular the procyanidine and catechins which are the most easily oxidised fractions; at the same time the intensity of colour of the musts increases though later absorbance at 420 nm decreases, finally producing wines of a lighter colour and a greater stability as indicated by the maderisation test. The effect of hyperoxygenation is detectable also by absorption at a wavelength of 320 nm due to the hydroxycinnamiltartaric acids which constitute the metabolites most involved in the enzymatic oxidation reactions: these are lower in the musts and especially in the wines produced using hyperoxygenation techniques. It is interesting to note that the addition of sulphur dioxide at the fermentation phase does not bring about great differences in the composition and stability of the phenols in the wine, the most notable effect due to the sulphur dioxide was, as could be predicted, on the amount of acetaldehyde which is rather limited in the batches fermented in the absence of the antiseptic (8 mg/L).
Finally, in regard to the microbiological aspect, some tests (FERRARINI *et al.*, 1991*c*) showed a reduction of 80-90 % in the quantity of levuliforms due to the separation effect of flotation thus permitting a more rational use of selected yeasts.

Conclusions

At the present time flotation is used with success in the clarification of grape musts. The interest it arouses at an industrial level is linked to its continuous affectiveness in plants with large potentials; however this separation technique can also be used with discontinuous batch systems and even applied in small sized firms. The parameters and conditions necessary to optimise the effects were determined experimentally; in particular with flotation it is possible to guide the liquid-solid separation up to the degree of limpidity desired, rationalizing the use of fining agents, and bringing about the stabilisation of phenols by means of the hyperoxygenation of the must.
The clarification of musts using flotation is particularly flexible even though in each case it is evaluated for the specific applications. The separative efficacy of flotation can be used in combination with other techniques for the realisation of new processes and products.

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FIGURES

Fig. 1 - Representation of the distribution of the solids in a circular flotation tank of a 200 hL/h plant: the scheme refers to a section taken through the centre, the curves identify regions with similar concentrations of solids (musts used: the drained must of Garaganege cultavar grapes; solids suspended in the raw must: 6% w/v; fining agents: 5 g/hL di gelatine, 30 g/hL bentonite and 50 g/hL silica sol, temperature of must 21°C).

Fig. 2 - Effect of the ratio volume/surface area (m^3/m^2) on the percentage of lees separated: flotation tests carried out with cv Garganega grape musts at 20 °C using 5 g/hL bentonite after enzymatic treatment for 2 hours with 1 g/hL pectolytic enzymes.

Fig. 3 - Yields from the flotation separation process of cv Garganega grape musts at 20°C as a function of the amount of suspended solids (centrifugation at 3000 rpm × 5') and the resting time. Fining agents used after enzymatic treatment (1 g/hL pectolytic enzymes for 2 hours): 5 g/hL gelatine, 40 g/hL bentonite and 50 g/hL silica sol.

Fig. 4 - Amount separated (limpid volume) during the course of the flotation process. Tests carried out on cv Garaganega grape musts at 20°C treated with enzymes (1g/hL pectolytic enzymes for 2 hours) and fining agents added (5 g/hL gelatine, 40 g/hL bentonite and 50 g/hl silica sol).

Fig. 5 - Separation of the cleared must from the flotation lees. Tests carried out on flotation lees of clarified cv Garaganega at 20°C grape musts (5 g/hL gelatine, 40 g/hL bentonite, 50 g/hl silica sol) after enzymatic treatment (1g/hL pectolytic enzymes for 2 hours) by means of flotation (flotation time = 30').

Fig. 6 - Effect of time of enzyme treatment on the yield of the flotation process: tests carried out at 20°C on cv Garaganega grape musts with 2 g/hL pectolytic enzymes and then subjected to flotation with the use of fining agents (5 g/hL gelatine, 40 g/hL bentonite, 50 g/hl silica sol).

Fig. 7 - Effect of the amount of pectolytic enzyme on the separation using flotation (flotation time = 15'): test carried out on cv Garganega grape musts to which fining agents were added (5 g/hL gelatine, 40 g/hL bentonite, 50 g/hl silica sol), subjected to enzymatic treatment for 2 hours at 20°C with increasing amounts of pectolytic enzymes.

Fig. 8 - Effect of phenolic substances on the flotation process: tests carried out at 20°C on cv Trebbiano grape musts to which increasing amounts of tannin were added and then clarified (10 g/hL di gelatine, 30 g/hL di bentonite and 80 g/hL silica sol) by means of flotation following enzymatic treatment (1g/hL pectolytic enzymes, resting time = 1 hour).

Fig. 9 - Effects of different clarifying treatments on the flotation process: tests carried out at 20°C on cv Chardonnay grape musts with 1 g/hL pectolytic enzymes for 1 hour and then subjected to flotation with different fining treatments as well as different combinations types and quantities.

Fig. 10 - Syneresis effect during flotation. The diagram represents separation by means of flotation carried out at 20°C in a cylinder with a resting time of 60 minutes and using fining agents (5 g/hL gelatine, 40 g/hL bentonite, 50 g/hl silica sol), after enzyme treatment (1 g/hL pectolytic enzymes for 2 hours); fractions: A = flotation lees, B = clear must (NTU<10) obtained by the syneresis effect, C = clear must (NTU ~ 50) due to the clarifying effect.

Fig. 11 - Trends in the consumption of oxygen at 20°C by musts from different cultivars.

Fig. 12 - Trends of total polyphenols, catechins, O.D. 320 nm and O.D. 420 nm during the hyperoxygenation tests on cv Garganega grape musts (m = average of 7 repeats; t*= Student constant per p = 0,05; s = standard deviation). Experimental conditions: gas = oxygen, pressure = 1 bar, temperature = 20 °C.

Fig. 13 - Amounts of polyphenols and catechins during hyperoxygenation tests carried at different concentrations of oxygen (1 and 5 bars additional pressure of oxygen) on cv Garganega grape musts (data average of 7 pereated tests . Experimental conditions: temperature = 20 °C.

Fig. 14 - Trends of total polyphenols and catechins during hyperoxygenation tests carried out at different temperatures (7, 20 and 35 °C) on cv Garganega grape musts (data average of 7 repeated tests). Experimental conditions: gas = oxygen, pressure = 1 bar.

Fig. 15 - Trends of total polyphenols and catechins (average of 7 repeated tests) during hyperoxygenation tests carried out on cv Garganega grape musts with or without the addition of fining agents (amounts of fining agents = 10 g/hL di gelatine + 40 g/hL bentonite + 60 g/hL silica sol). Experimental conditions: gas = oxygen, pressure= 1 bar, temperature = 20 °C.

TABLES

Tab. 1 - Effect of pectolytic emzymes (1g/hL commercial preparation, 2 hours of enzymatic treatment at 20°C) on flotation carried out with the addition of fining agents (5 g/hL di gelatine, 40 g/hL bentonite and 50 g/hL silica sol).

Tab. 2 - Comparison between different pectolytic enzyme preparations used in the treatment of musts before flotation carried out at 20°C with the addition of fining agents(5 g/hL gelatine, 40 g/hL bentonite and 50 g/hL silica sol): analysis of variance at one factor (enzymatic preparation) with repeats (n=5), comparison of the means using Tuckey's test. Similar letters indicate means that are statistically not different by p= 0.05 (small letters) and p = 0.01 (capital letters).

Tab. 3 - Measurement of the superficial charge (Z potential at 20°C) and some analytical parameters of cv Garganega musts: A - must from the first pressing, B - must from the last pressing.

Tab. 4 - Effect of hyperoxygenation on some components of musts and the relative wines: tests on sedimentation and flotation carried out with similar doses of enzymes and fining agents (1 g/hL pectolytic enzymes, 5 g/hL gelatine, 40 g/hL bentonite); static sedimentation was carried out without heating for a period of 24 hours, the flotation treatment was carried out 3 hours after pressing at 22°C.

Tab. 1

		ENZYME TREATED	ENZYME UNTREATED	F (Fischer Test)
LEES	%	31	38	12.71**
TURBIDITY	NTU	35	145	5.02*
TOTAL NITROGEN	mg/L	346	433	10.11**
TOTAL PHENOLS	mg/L	534	725	8.29*
CATECHINS	mg/L	9	11	53.13**
Repeats	n	6	9	

Tab. 2

ENZYMATIC PREPARATION	n	$\bar{x}$	
		TURBIDITY (NTU)	LEES (% w/v)
I	5	41.0 A	31.0 C
II	5	50.0 C	24.0 AB
III	5	43.8 B	22.0 A
IV	5	48.0 C	22.0 A
V	5	48.0 C	23.0 AB
VI	5	50.0 C	23.6 AB
VII	5	41.8 AB	24.0 AB
VIII	5	48.0 C	25.0 B
F (Fischer Test)		58.19**	32.35**

Tab 3

		A	B
Superficial charge	mV	+ 5.7	- 14.0
Total phenols	mg/L	318	2300
Procyanidins	mg/L	30	62
Catechins	mg/L	20	113
Turbidity	NTU	700	6000

Tab. 4

ANALYSIS OF MUSTS	SEDIMENTATION	FLOTATION	FLOTATION WITH HYPEROXYGENATION	
SO_2 during clarification mg/L	0	50	0	
O.D. 320 nm	12.40	9.88	10.72	
Total phenols mg/L	686	589	595	
Procyanidins mg/L	94	41	16	
Catechins mg/L	36	20	6	
ANALYSIS OF WINES				
SO_2 during fermetation mg/L	50	50	50	0
pH	3.18	3.04	3.23	3.27
Ashes g/L	2.73	2.73	2.69	2.68
Alkalinity of ashes meq/L	25.4	26.8	28.2	27.2
Total SO_2 mg/L	72	68	71	75
Acetaldehyde mg/L	29	38	24	8
Total phenols mg/L	375	278	222	251
Procyanidins mg/L	59	26	11	10
Catechins mg/L	37	16	7	6
O.D. 320 nm	3.95	3.16	2.50	2.50
O.D. 420 nm × 1000	101	110	100	108
Incr. % O.D.420 nm 50°C×48h	44	20	16	9

Fig. 1

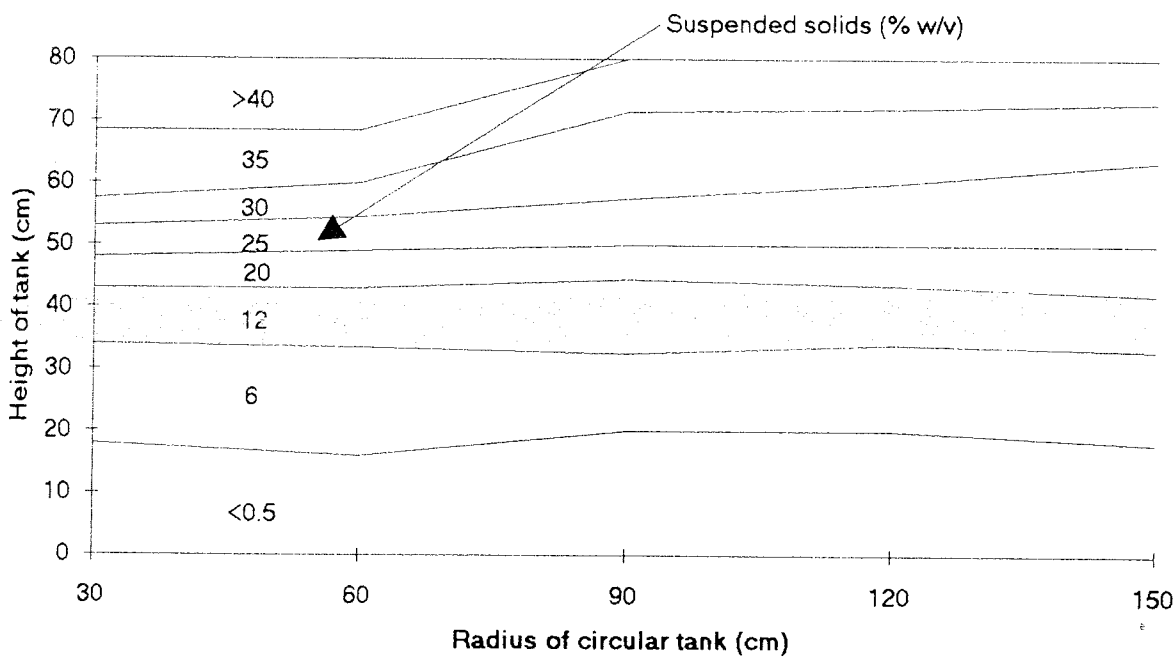

Suspended solids (% w/v)
80
70
60
50
40
30
20
10
0
>40
35
30
25
20
12
6
<0.5
Height of tank (cm)
30
60
90
120
150
Radius of circular tank (cm)

Fig. 2

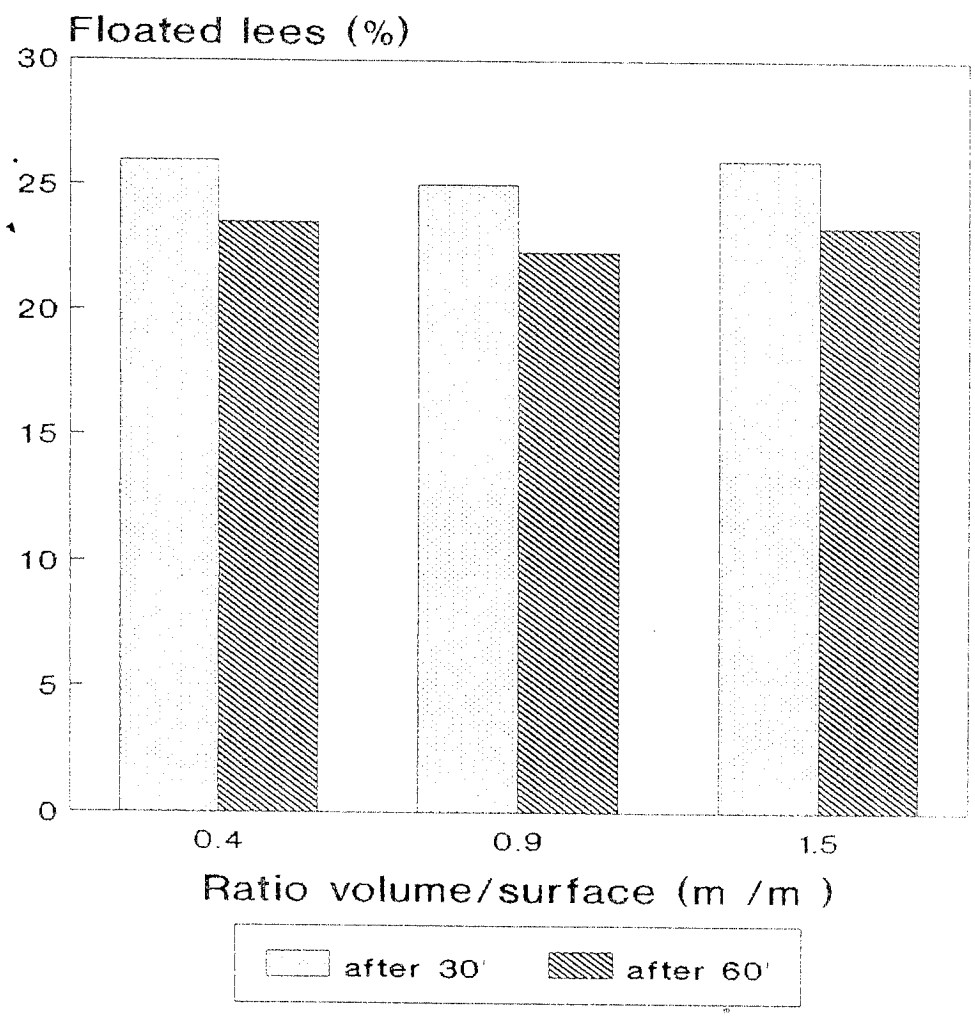

Floated lees (%)
30
25
20
15
10
5
0
0.4
0.9
1.5
Ratio volume/surface (m /m)
after 30'
after 60'

Fig. 3

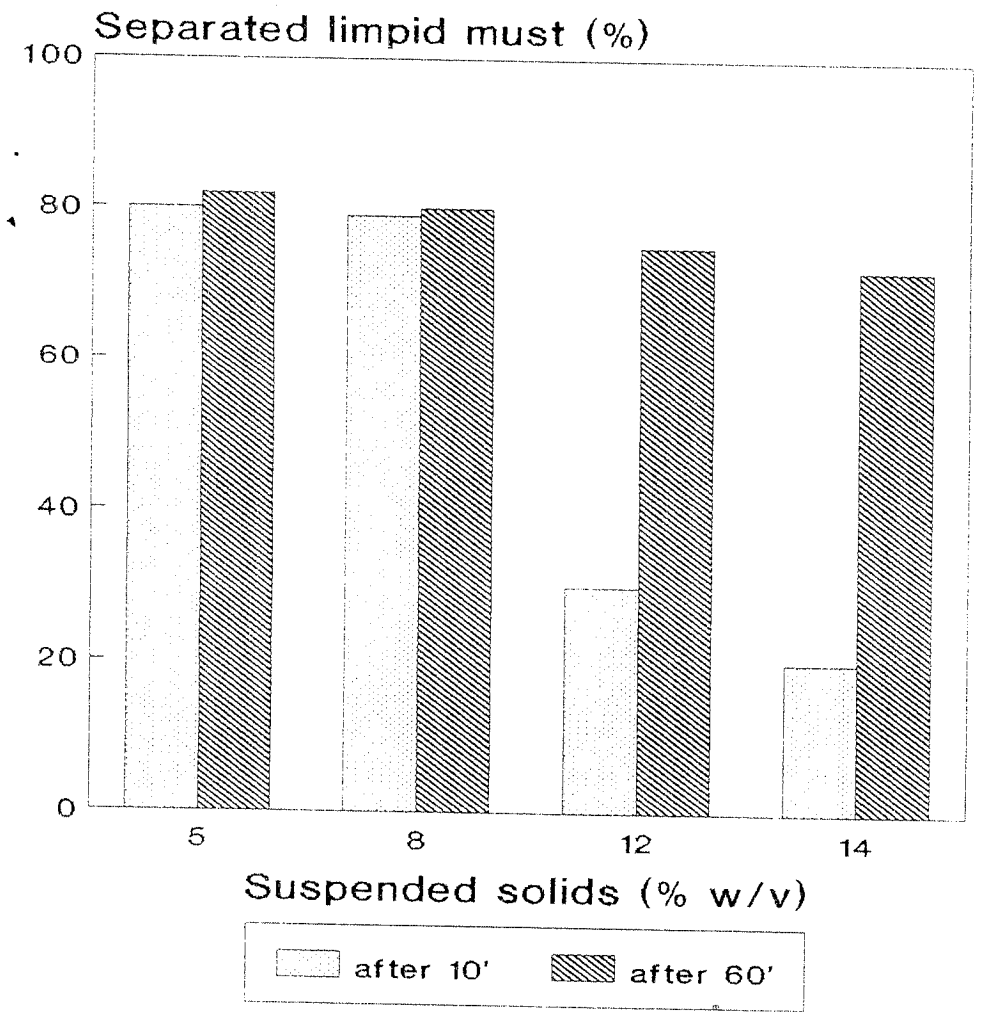

Separated limpid must (%)
100
80
60
40
20
0
5
8
12
14
Suspended solids (% w/v)
after 10'
after 60'

Fig. 4

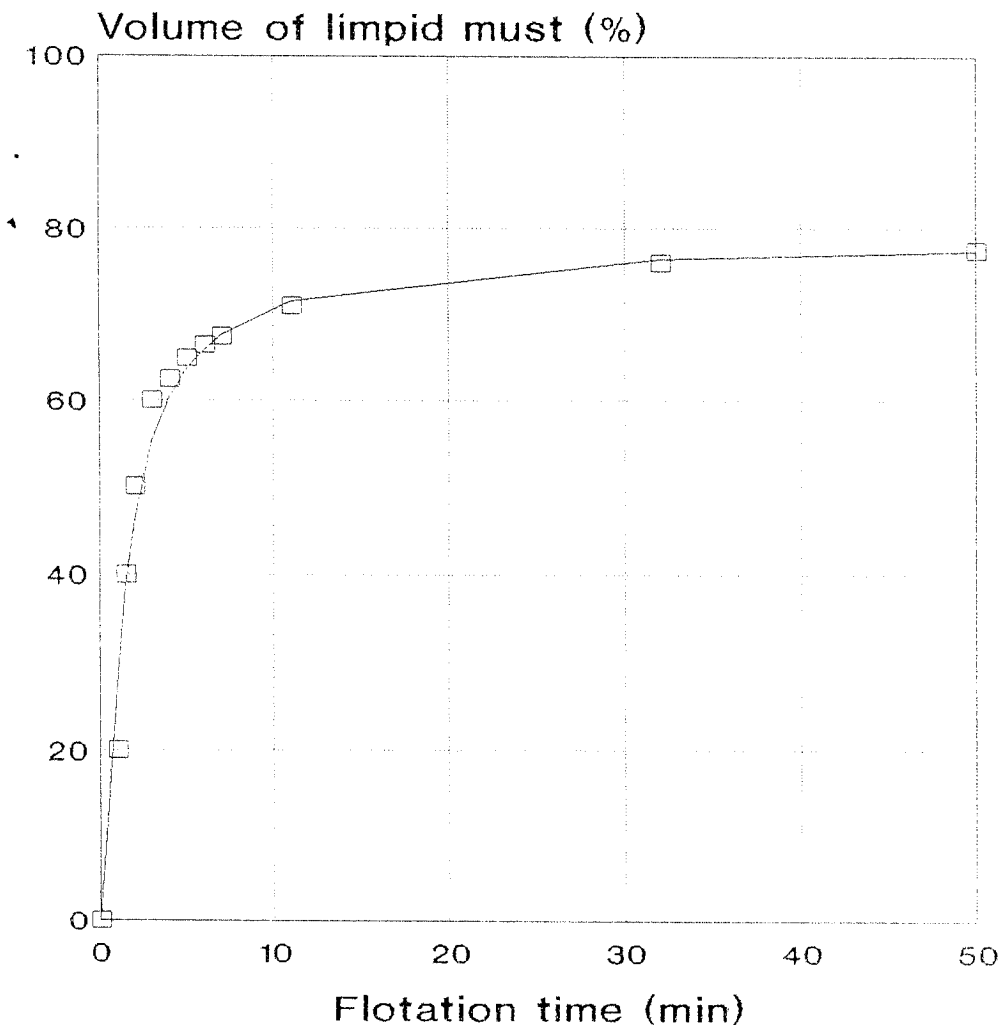

Volume of limpid must (%)
100
80
60
40
20
0
0
10
20
30
40
50
Flotation time (min)

Fig. 5

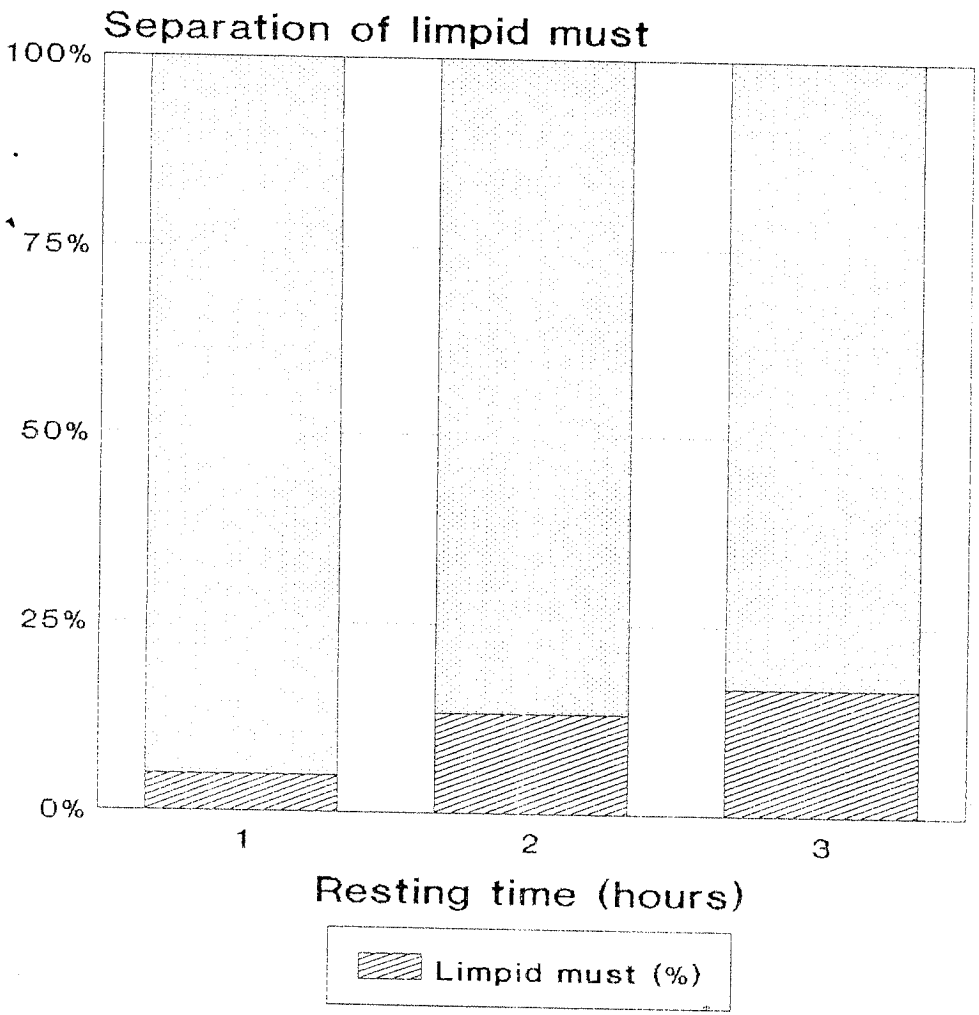

Separation of limpid must
100%
75%
50%
25%
0%
1
2
3
Resting time (hours)
Limpid must (%)

Fig. 6

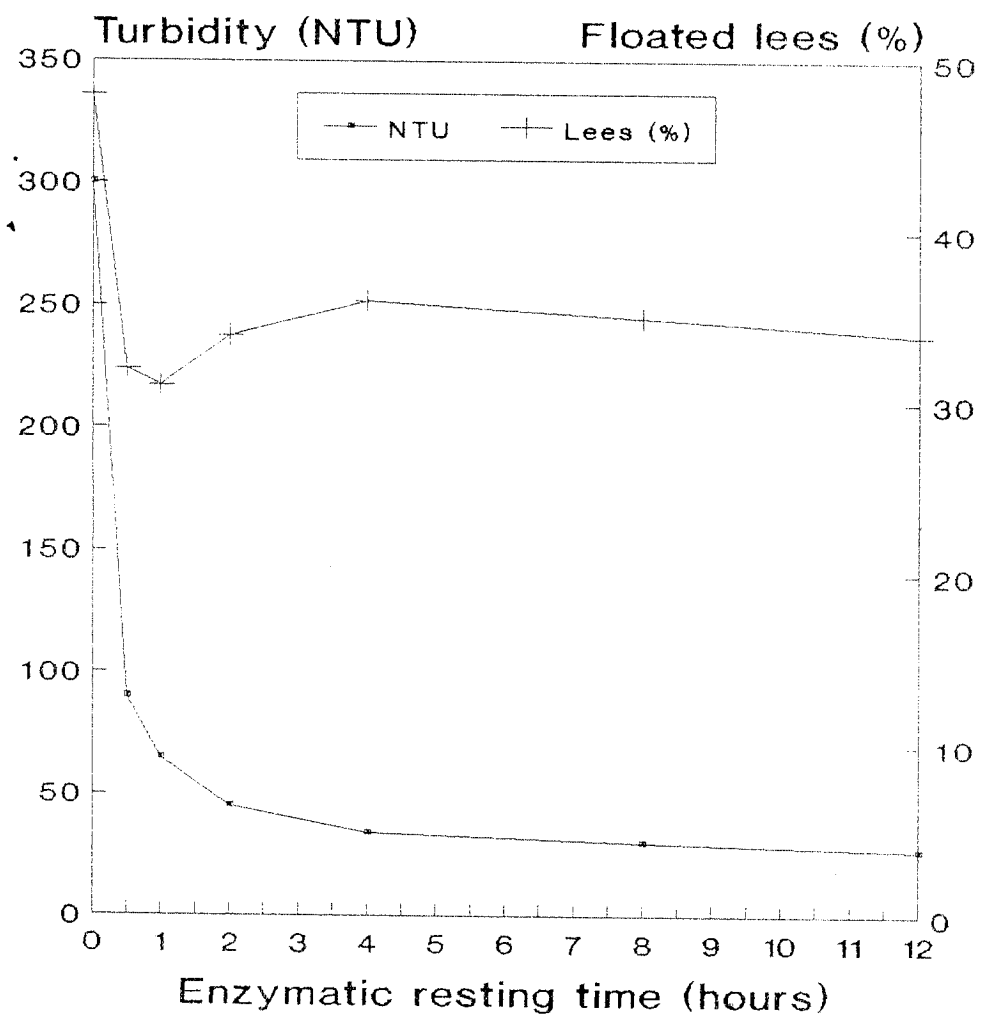

Turbidity (NTU)
Floated lees (%)
NTU
Lees (%)
350
300
250
200
150
100
50
0
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12
Enzymatic resting time (hours)

Fig.7

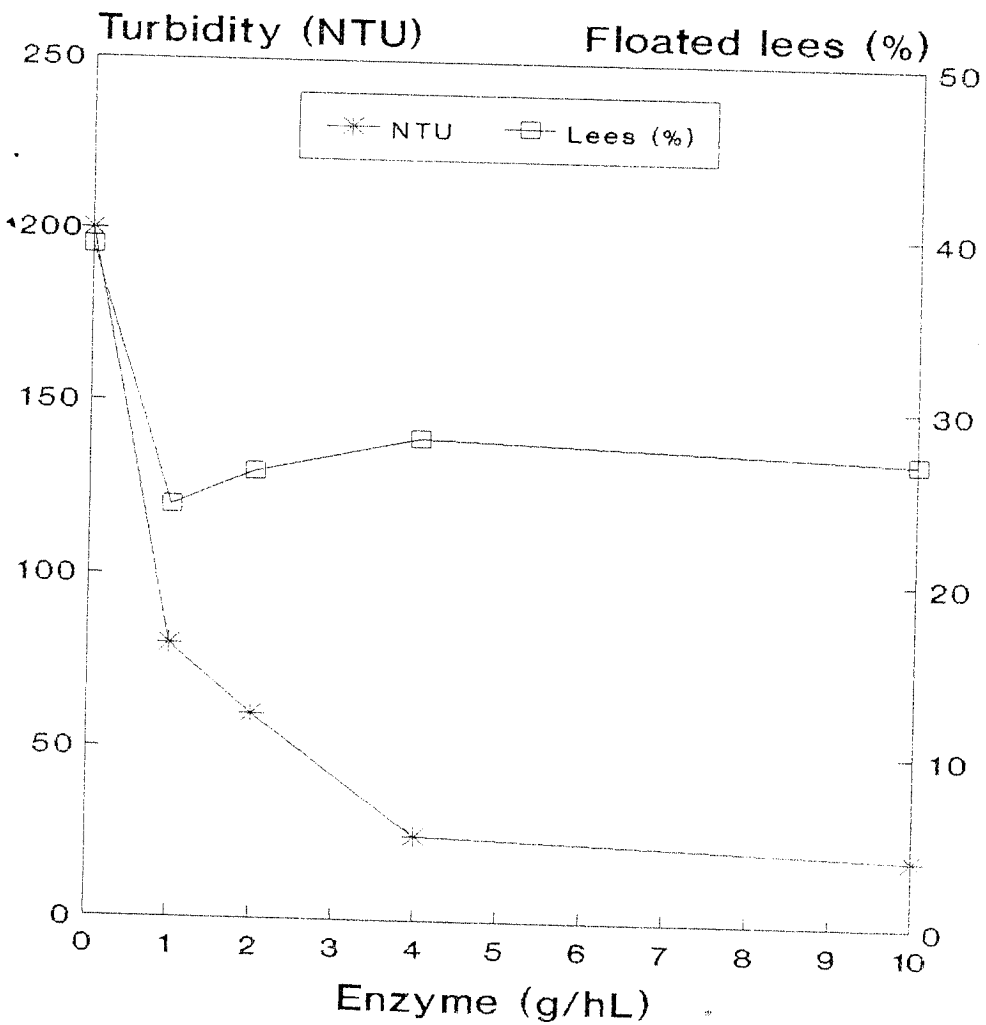

Turbidity (NTU)
Floated lees (%)
NTU
Lees (%)
250
200
150
100
50
0
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10
Enzyme (g/hL)

Fig.8

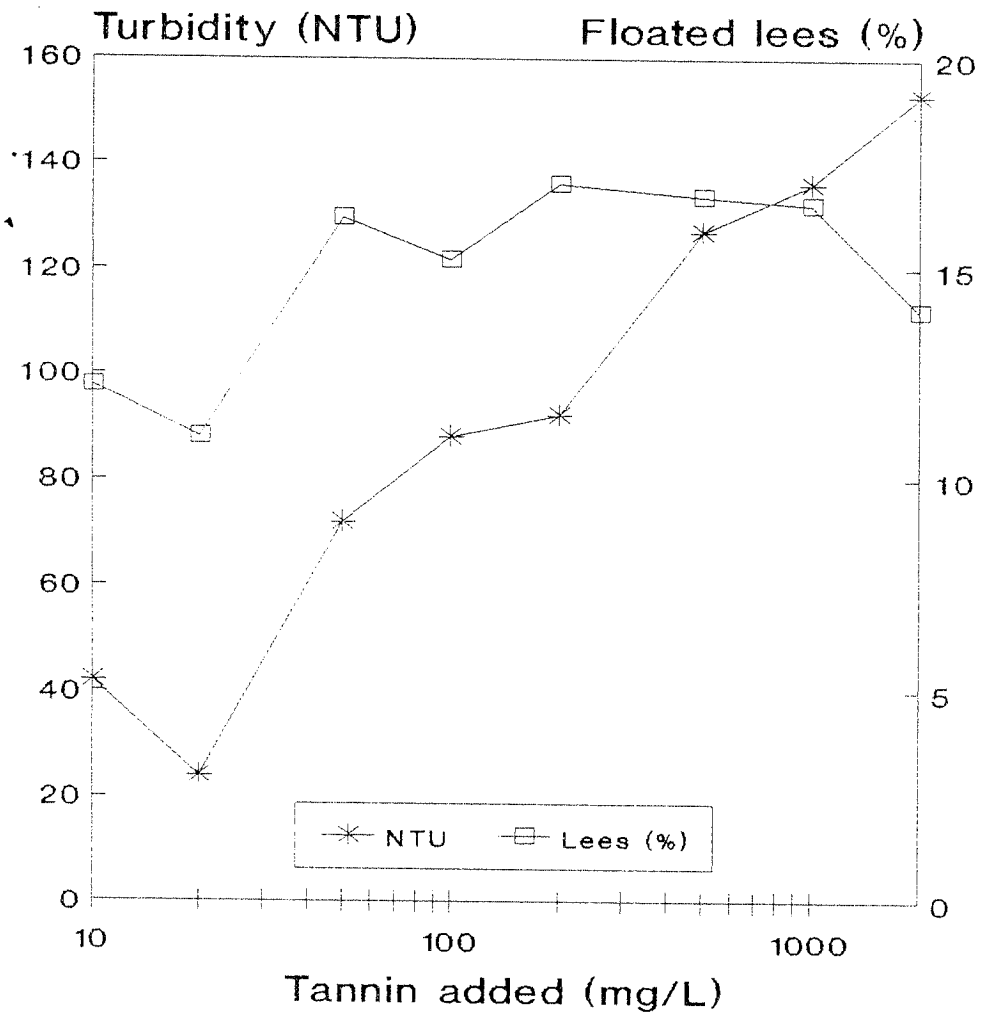

Turbidity (NTU)
Floated lees (%)
160
140
120
100
80
60
40
20
0
20
15
10
5
0
NTU
Lees (%)
10
100
1000
Tannin added (mg/L)

Fig. 9

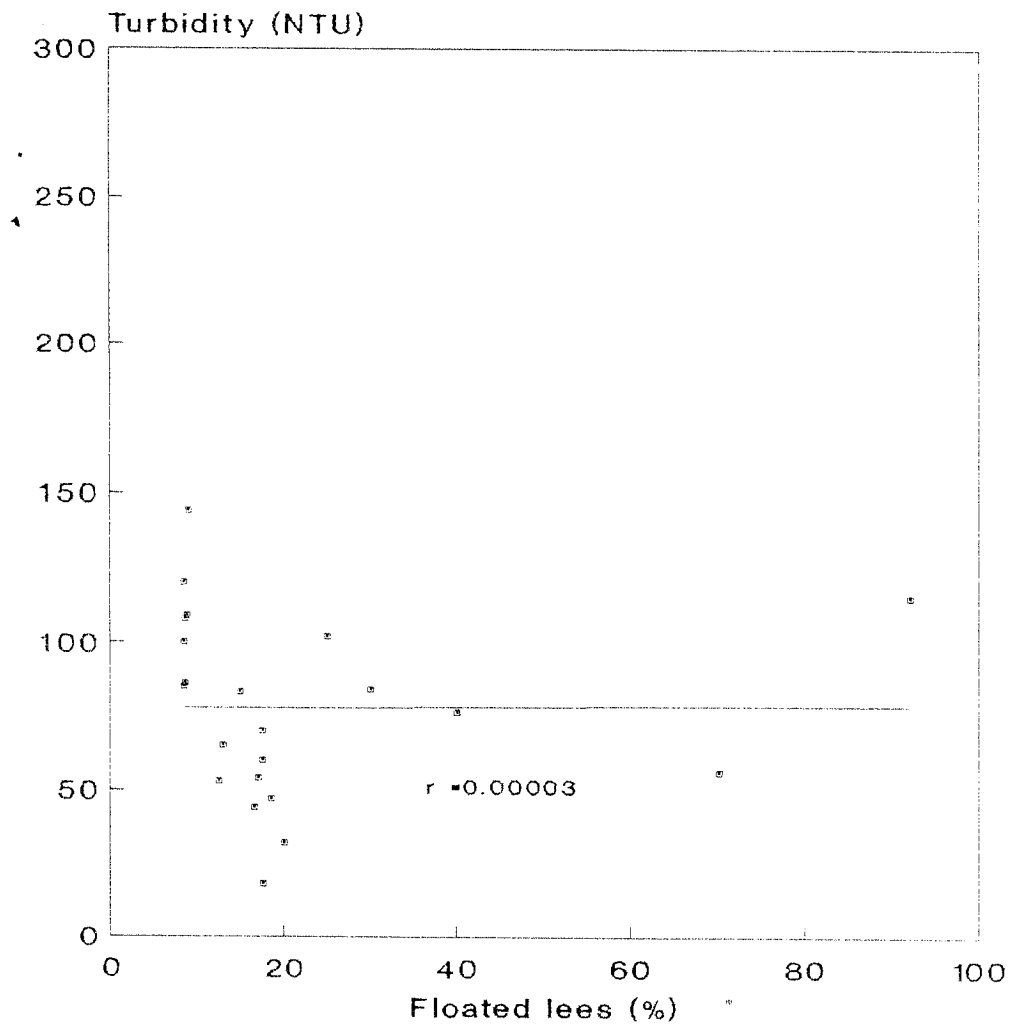

Turbidity (NTU)
300
250
200
150
100
50
0
0
20
40
60
80
100
Floated lees (%)
r =0.00003

Fig. 10

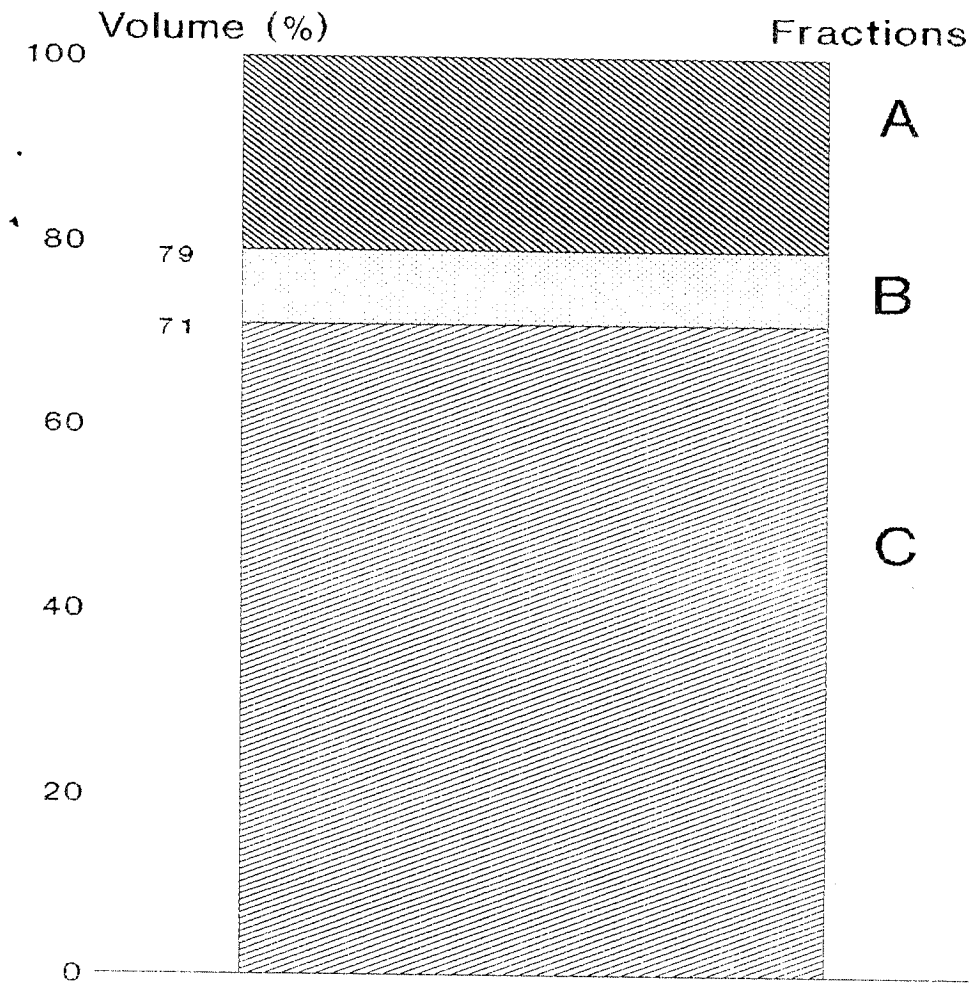
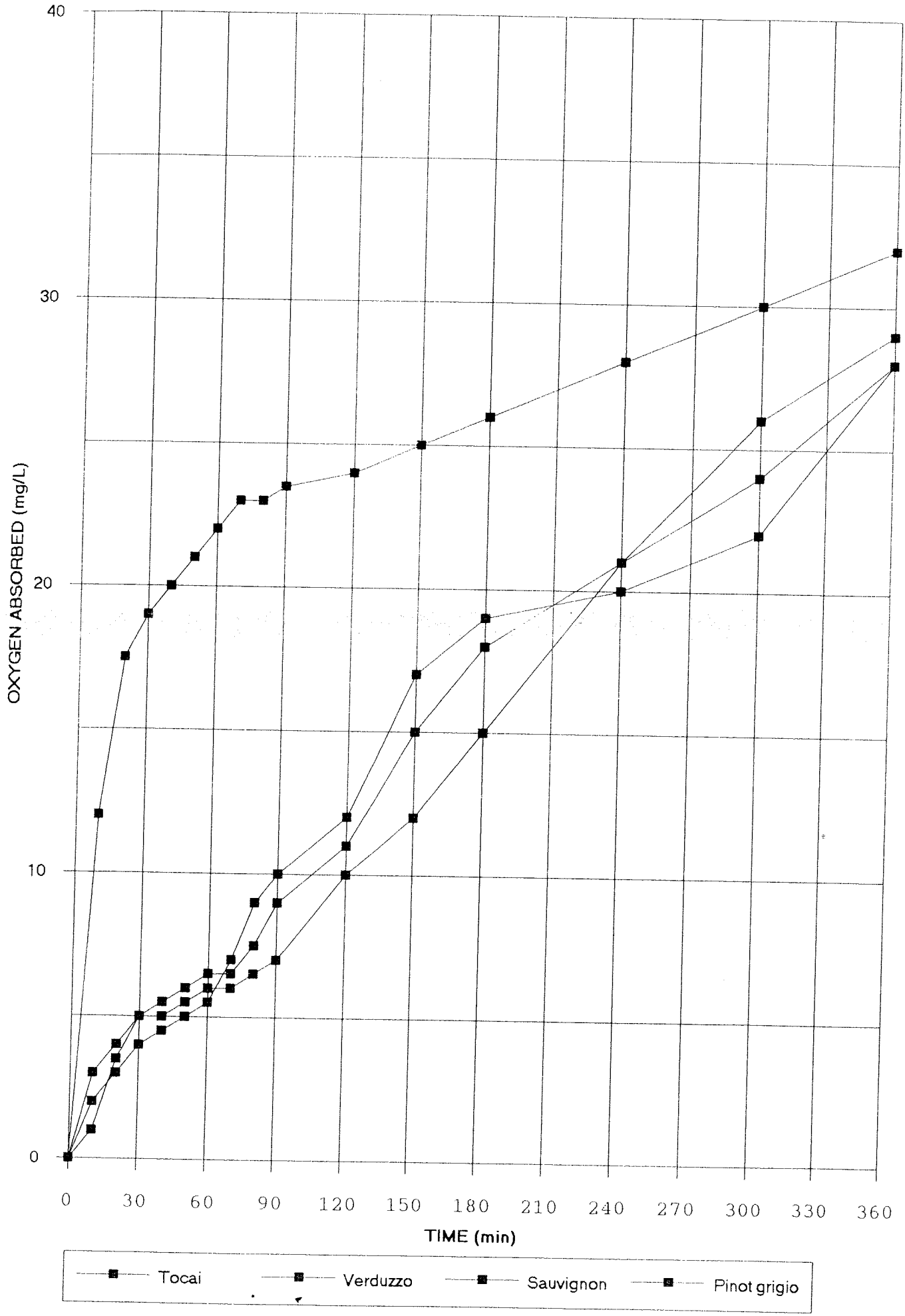
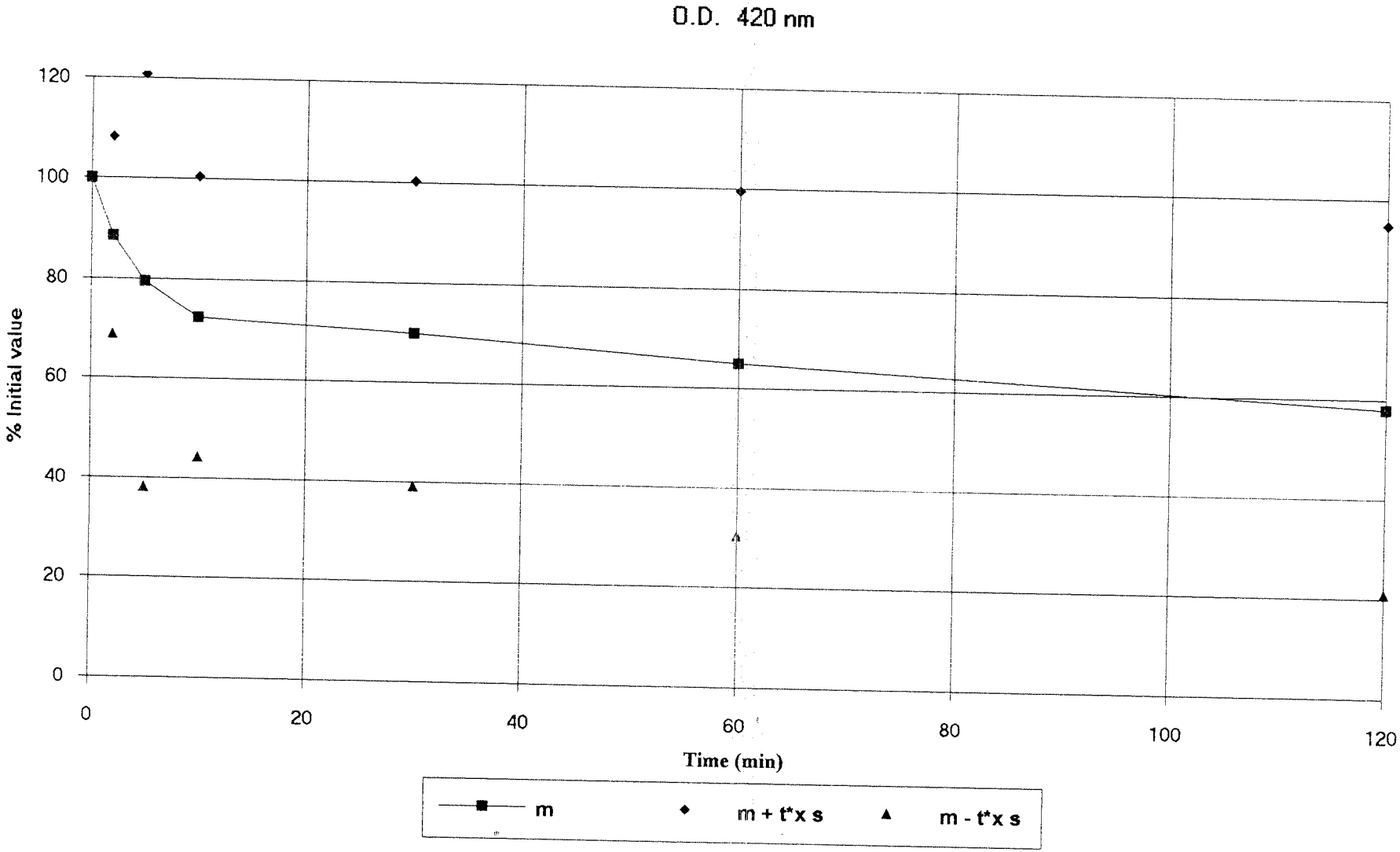


FIG. 11


40
30
20
10
0
OXYGEN ABSORBED (mg/L)
0
30
60
90
120
150
180
210
240
270
300
330
360
TIME (min)
Tocai
Verduzzo
Sauvignon
Pinot grigio


O.D. 420 nm
% Initial value
120
100
80
60
40
20
0
0
20
40
60
80
100
120
Time (min)
m
m + t*x s
m - t*x s

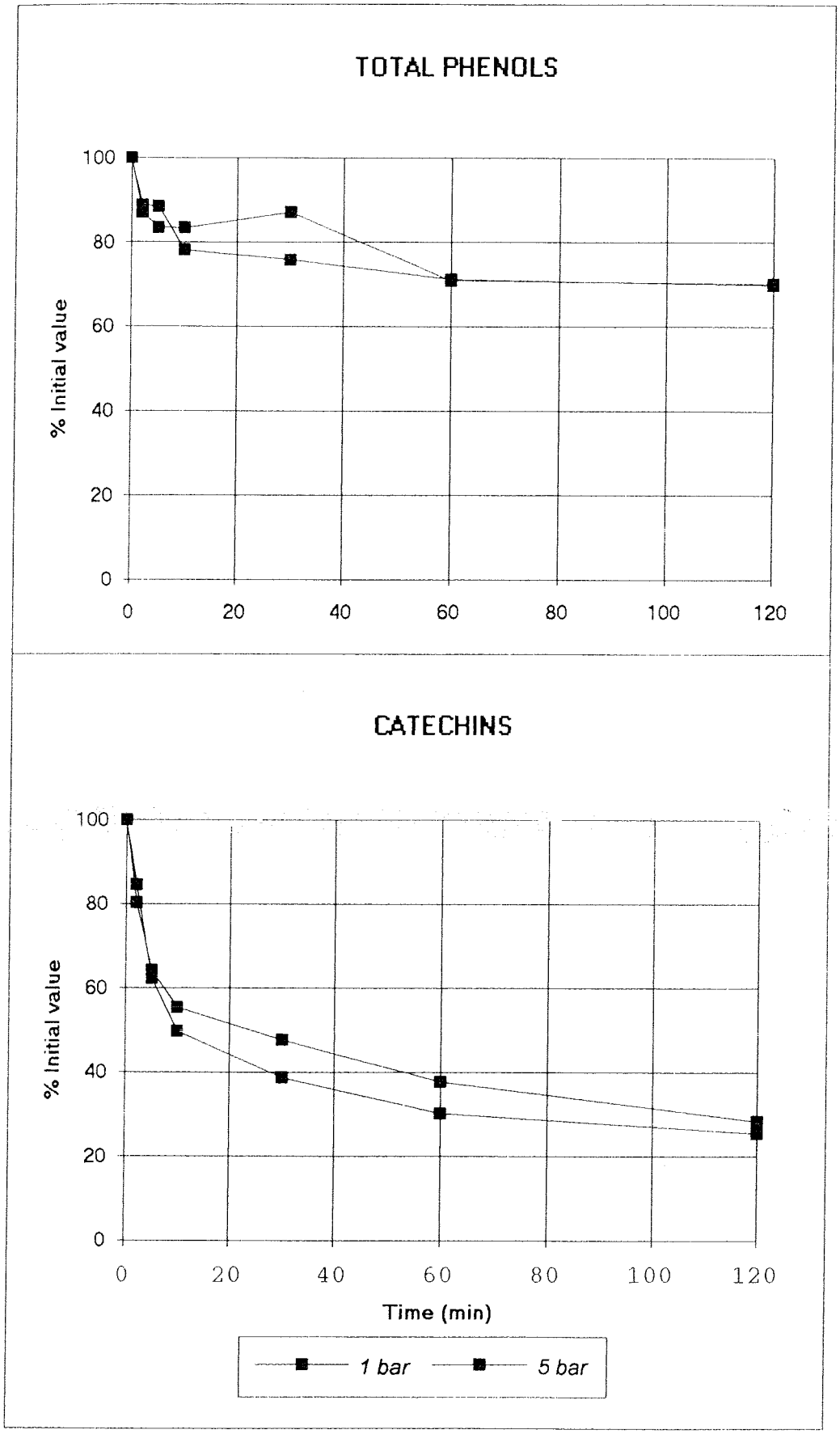

TOTAL PHENOLS
% Initial value
100
80
60
40
20
0
0
20
40
60
80
100
120
CATECHINS
% Initial value
100
80
60
40
20
0
0
20
40
60
80
100
120
Time (min)
1 bar
5 bar

Fig. 13

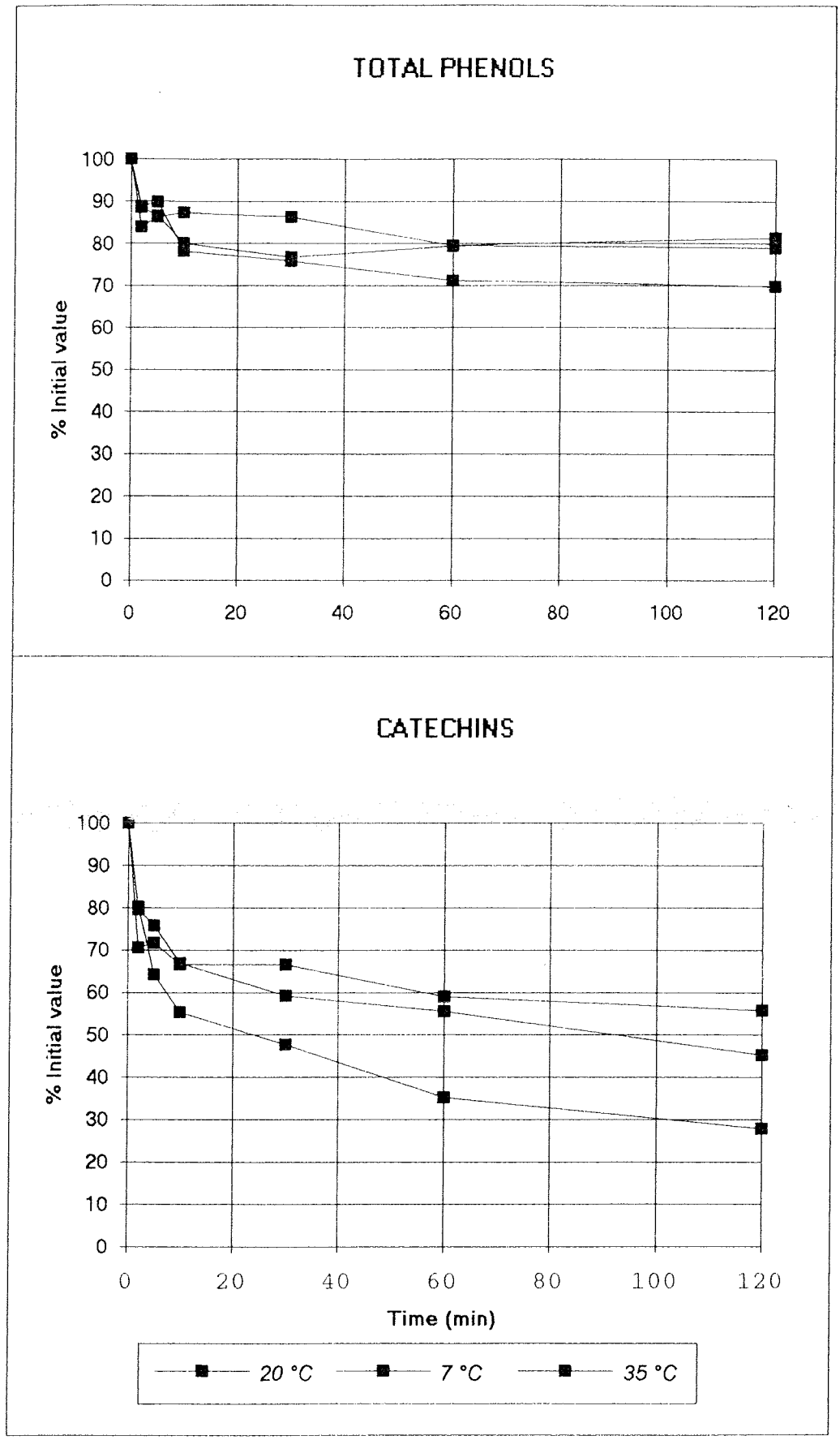

TOTAL PHENOLS
% Initial value
100
90
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
120
CATECHINS
% Initial value
100
90
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
120
Time (min)
20 °C
7 °C
35 °C

Fig. 14

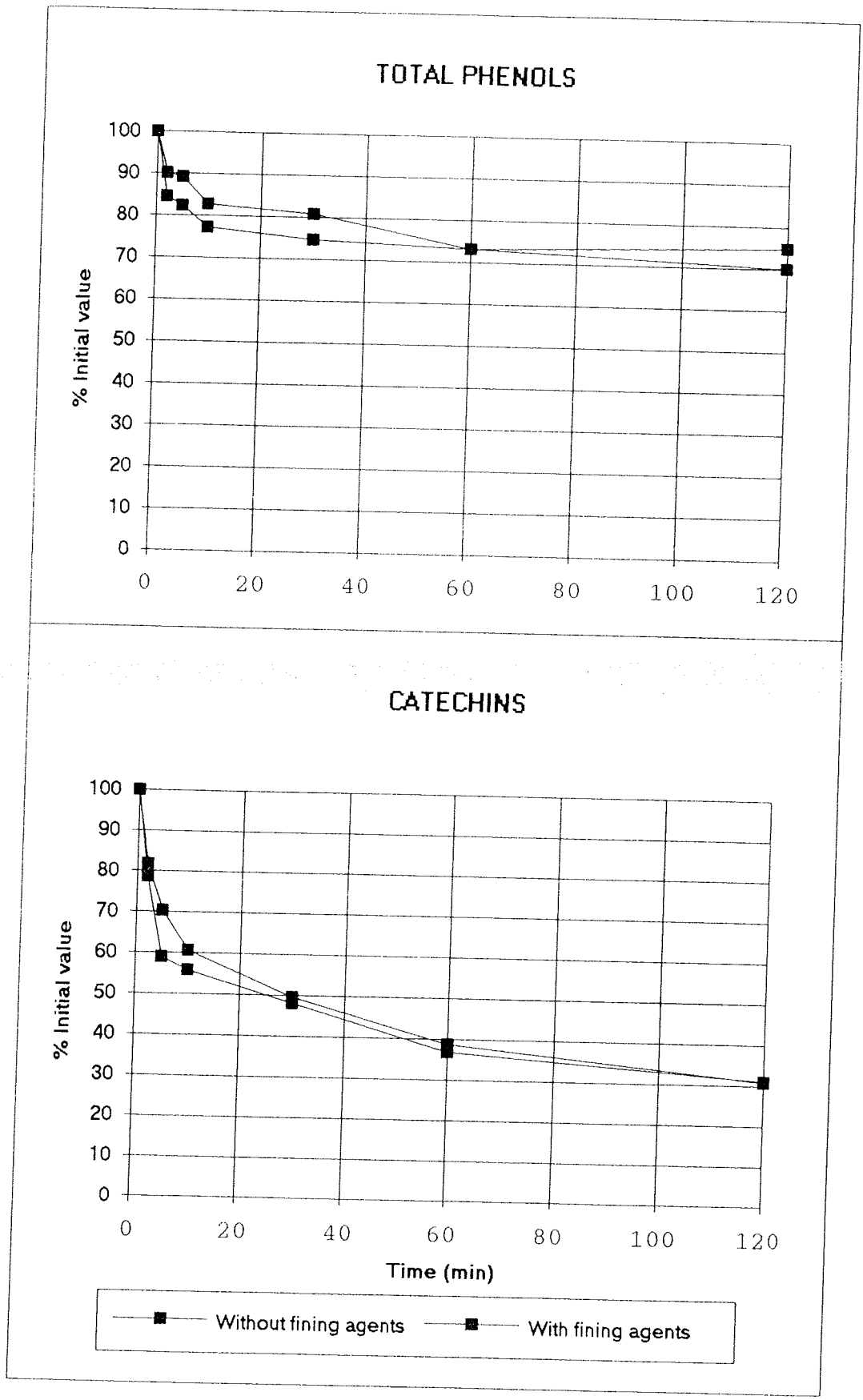

TOTAL PHENOLS
% Initial value
100
90
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
120
CATECHINS
% Initial value
100
90
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
120
Time (min)
Without fining agents
With fining agents

Fig. 15

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