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# Inhibition by yeast strain of tyrosinase activity in must and wine

by

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**Inhibition par la souche de levure de l'activité de la tyrosinase du moût et du vin**

Résumé. — Le cours de l'activité de la tyrosinase dans les moûts est rélié à la souche de levure employée: la souche 633 rend l'activité pratiquement inexistante pendant les premiers jours de son développement. D'autres souches provoquent une baisse drastique pendant les premières 48 heures, suivie par un récouvrement de l'activité de la tyrosinase pendant la phase exponentielle de leur développement en corrélation à une chute substantielle des composés phénoliques totaux et à la hausse exponentielle de la couleur. Le pouvoir inhibiteur sur la tyrosinase *in vitro* est un caractère diffusé des lèvures bien que quantitativement différent; ce caractère n'est pas toujours associé à la stabilité de la couleur des vins blancs. Tel caractère, possedé d'une façon remarquable par les souches 633 et 36, est lié à la biosynthèse, même dans les substrats synthétiques, d'un composé capable de se lier aux unités protéiques de l'enzyme, de façon à prévenir l'activité enzymatique.

## Introduction

Among the factors affecting the stability of wine colour, the strain of yeast, bringing about the alcoholic fermentation, certainly plays a primary role. Šafar (1971—1972) reports that wines obtained from fermentation with strains of the *Saccharomyces carlsbergensis*, *Saccharomyces vini* and *Saccharomyces rosei* species, are usually more stable than those fermented with other species of yeast. During fermentation, the above mentioned strains produce $SO_2$. The stabilizing power is a rarely encountered character of *Saccharomyces cerevisiae*: in an earlier work (Zambonelli *et al.* 1972) it has been shown that, out of 70 strains tested, only two possessed this character, which in all cases, however, appeared to be associated with the production of $SO_2$, always bound, in amounts greater than 1.5 meq/l. However, a direct relationship between the $SO_2$ produced and the degree of stabilization does not always exist, since some strains, producing substantial amounts of $SO_2$, have not been able to prevent browning. On the other hand, Šafar (1971—1972) reports that a strain of *Saccharomyces carlsbergensis* produces unimportant amounts of $SO_2$ at the same time giving stable wines.

The object of this research was to investigate into any other possible factor, besides the production of $SO_2$, involved in the stabilizing action of some strains. The polyphenoloxidase enzymes (PPO) are no doubt the browning-inducing agents that have been more thoroughly and widely discussed and studied. The PPO enzymes present in the must may originate from different sources, i.e. the tyrosynase (E.C. 1.10.3.1.) present in grapes and the tyrosinase and the laccase (E.C. 1.10.3.2.) produced by fungi, by *Botrytis cinerea* in particular, the importance of which in the musts from *Botrytis*-affected grapes has been emphasized by Dubernet and Ribéreau-Gayon (1973, 1974). Ascomycetes secrete different kinds of laccase with varying molecular weight and different specificity with respect to polyphenols; their quantitative ratio depends on the genetic information and on environmental conditions (Molitoris and Esser 1971, Schánel and Esser 1971).

Bearing in mind that the primary role of oxidase enzymes in the browning of white wines may be a questionable matter, the present discussion concerns the direct *in vivo* and *in vitro* action of strains stabilizing colour on the tyrosinase enzyme of must and wine.

## Material and Methods

Microorganisms: Wild strains of *Saccharomyces cerevisiae*, belonging to the collection of the Istituto di Microbiologia e Industrie Agrarie of the University of Bologna, have been used for these tests. Tests have also been carried out using 5 strains kindly supplied by Prof. G. WÜRDIG (strains 25 and 58: *Saccharomyces uvarum*, 26 and 59: *Saccharomyces carlsbergensis*, 27: *Saccharomyces cerevisiae*, 36: *Saccharomyces bayanus*, and strain 9080 A.T.C.C.: *Saccharomyces carlsbergensis*). A wild strain of *Botrytis cinerea* kindly supplied by the Istituto di Patologia Vegetale of the University of Bologna has been employed for the production of laccase.

Nutritional conditions: The synthetic substrate used for yeasts has already been described by ZAMBONELLI and GUERZONI (1970). The *Botrytis* strain has been grown in the substrate suggested by GRABBE *et al.* (1968). Laboratory fermentations have been carried out in 200 ml vessels with filtered and centrifuged musts from healthy Albana grapes (1974), following fermentation on the basis of $CO_2$ loss by weighing, according to the method described by CASTELLI (1960).

Biological materials: The fungus tyrosinase (grade III) produced by Sigma Chemical Company, St. Louis Mo., has been used for inhibition tests *in vitro* on tyrosinase activity. Inhibition tests on grape tyrosinase have been carried out using musts from carefully selected, healthy grapes. Such tests have been conducted directly on the must without prior precipitation or purification of the enzymes, after the absence of laccase had been ascertained.

Enzymic reactions: The enzymic determination of the total PPO activity has been made according to the following two methods: the method developed by BAYER *et al.* (1957) — using pyrogallol as a substrate —, and the method developed by KERTESZ *et al.* (1972) following the formation of dopachrome at 480 nm from 3,4-dihydroxyphenylalanine (DOPA) as a substrate. Using the method described by KERTESZ (1968), it has been calculated that an increase of the optical density at 480 nm of 0.001 corresponds to the formation of $2.7 \times 10^{-7}$ moles of dopachrome. The laccase activity has been determined on the basis of the formation of the yellow chromophore, absorbing at 436 nm, from potassium ferrocyanide, the latter being the substrate specific to laccase and not to tyrosinase, according to the method described by MOLITORIS and ESSER (1971).

Analytical methods: Electrophoresis. — The electrophoresis of tyrosinase has been performed on Whatman 3 MM paper, using the buffers reported by SHAW and PRASAD (1970). The enzyme has been visualized, staining with amidoblack or incubating in a solution of 0.1 M phosphate buffer, pH 6.8, containing 0.15% L-DOPA for several hours.

Paper chromatography. — The investigation into the compound(s) inhibiting the tyrosinase activity has been carried out throuh ascending chromatography on Whatman 3 MM paper, using the following eluents: (A) n-butanol/acetic acid/water (4 : 1 : 2); (B) 5% ammonia/acetone/acetic acid/n-butanol/water (3 : 5 : 3 : 7 : 0.2); (C) methanol/benzene/acetic acid (8 : 45 : 4). In order to show the inhibitor of the en-

zymic activity, chromatograms have been sprayed with a solution of tyrosinase enzyme (9 mg in 100 ml) and then with a solution of pyrogallol (500 mg in 100 ml). After some time, the areas of inhibition can be clearly seen on an uniform, yellow background resulting from the formation of purpurogallin. Total polyphenols calculated as tannic acid have been determined by Folin-Ciocalteu's reagent, following the method described by Sapis and Ribéreau-Gayon (1968).

The colour of wine was evaluated as optical density at 420 nm. $SO_2$, free and bound, has been determined alkali-metrically, following the method described by Paul (1958), after distillation.

Reproducibility of results: All experiments were carried out in duplicate. Each experiment was repeated at least two more times.

## Results

### Changes in the tyrosinase activity during fermentation with different strains of yeast

The colour of wine was evaluated as optical density at 420 nm. $SO_2$, free and by Kertesz (1968) and using 3,4-dihydroxyphenylalanine as a substrate, in two duplicate fermentation experiments on Albana must from 1974 healthy grapes. The 3,4-dihydroxyphenylalanine is a substrate of both tyrosinase and laccase; in these musts, however, the laccase activity, determined by potassium ferrocyanide, appeared to be practically absent. The total PPO activity reported here, therefore, is related to tyrosinase only. The present tests concern the soluble activity only, in that the enzyme linked to the suspended particles had been removed by centrifugation after each sampling. Strain 633, *Saccharomyces cerevisiae,* which normally gives rise to stable wines, and two strains of *Saccharomyces carlsbergensis* (Nos. 59 and 9080), giving rise to wines susceptible to browning, have been used in these tests. The following parameters have been recorded daily during fermentation: rate of fermentation, tyrosinase activity, and total polyphenols. When fermentation was complete (it lasted 7 days with strains 633 and 59 and 8 days with strain 9080), wines have been centrifuged and exposed to air and light and the wine-browning has been recorded in addition to the above mentioned parameters. Figs. 1 a, 1 b and 1 c show the results of one fermentation test: apart from a slight difference in fermentation times, due to the amount of inoculum used, the behaviour of the parameters is almost the same in all the experiments. In Fig. 1 a, relating to strain 633, the line of the colour, determined as optical density at 420 nm, is parallel to the abscissa. In wines obtained by strain 59 (Fig. 1 b) and by strain 9080 (Fig. 1 c) during the 48 hours immediately following racking, an exponential rise in the colour has been recorded, which successively stabilizes. Slower browning processes are reported in the literature (taking even months): these differences are probably ascribable to both the kinds of musts used and to the time of racking (very early in our case).

When examining the different individual parameters, a drastic fall in phenols during the first 24 hours can be observed, followed by a slight recovery and stabilization during the following days of growth. During the 48 hours following racking, on the contrary, one notes a marked decrease in such compounds in the wines produced by strains 59 and 9080, but not in the wines produced by strain 633; in the latter, the absolute values of phenols are always higher. Such a decrease is accompanied by a browning process that shows an exponential rise during the first 24 hours after racking, as well as by a sudden reduction of the tyrosinase activity. This

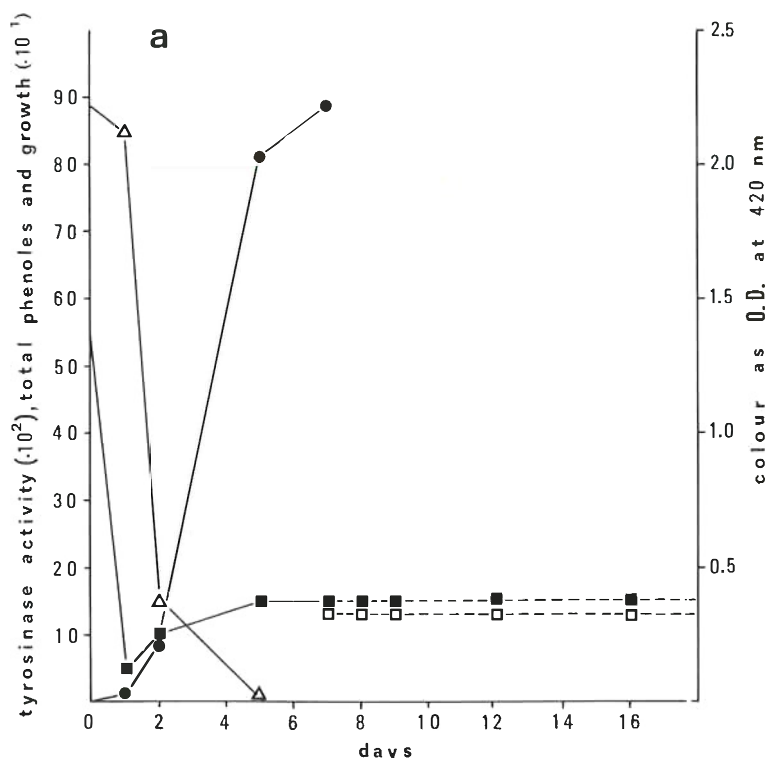


Fig. 1: Influence of the yeast strain on tyrosinase activity, total phenols and colour during and after fermentation: (a) strain 633; (b) strain 59; (c) strain 9080. Symbols: ——— = during fermentation; - - - - = after fermentation; ● = growth as $CO_2$ loss (g/100 ml of must); ■ = total phenols as tannic acid (mg/100 ml); △ = tyrosinase activity as dopachrome formed ($10^{-4}$ moles/ml/h); □ = colour as optical density at 420 nm.

Influence de la souche de levure sur l'activité de la tyrosinase, les composés phénoliques totaux et la couleur pendant et après la fermentation: (a) souche 633; (b) souche 59; (c) souche 9080. Symboles: ——— = pendant la fermentation; - - - - = après la fermentation; ● = vitesse de fermentation en $CO_2$ produit (g/100 ml de moût); ■ = composés phénoliques totaux calculés en acide tannique (mg/100 ml); △ = activité de la tyrosinase en dopachrome formé ($10^{-4}$ moles/ml/h); □ = couleur en densité optique à 420 nm.

activity has a different course during fermentation as a function of the strain used: with strain 633, the activity drastically falls during the first 48 hours, corresponding to the incubation phase of growth, while it decreases progressively, but more slowly, during the exponential phase and is not detectable at the beginning of the stationary phase. In strains 9080 and 59, a drastic drop always occurs during the first 48 hours, followed by a recovery during the exponential stage of development. At the time of racking, the tyrosinase activity is still important in these two strains, but a drastic fall is recorded accompanied by a logarithmic rise in colour and a decrease in total phenols.

The sudden fall in the enzyme during the first 24 hours of growth cannot reasonably be ascribed to the activity of yeast, the latter still being in its incubation phase of growth, but most probably to the fact that the enzyme is linked with the variety of oxidizable substrata present in the must which make it less available to the DOPA used for determining its activity. During the following hours, a dif-

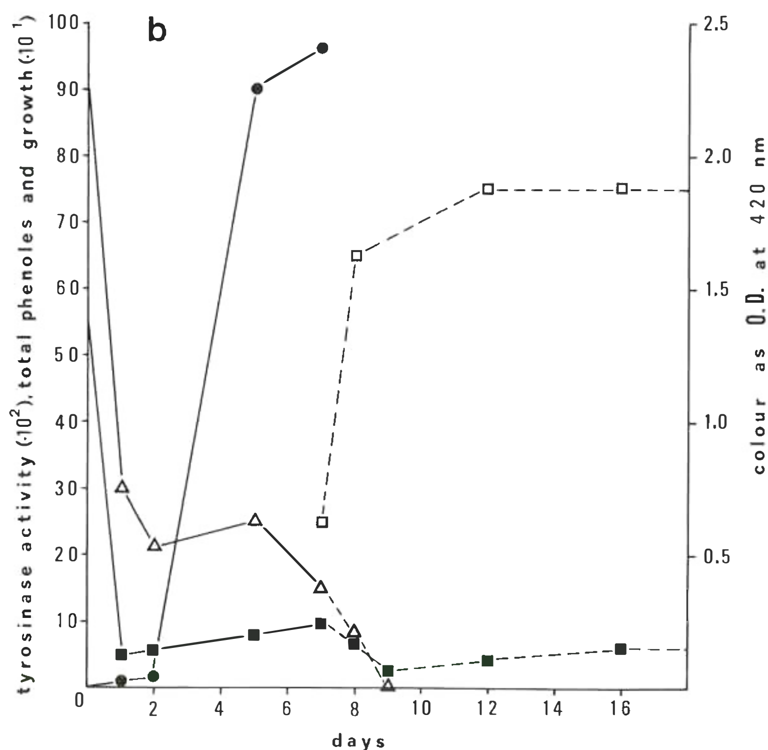


Fig. 1 b: Legend see Fig. 1 a.
Voyez la légende de Fig. 1 a.

ferentiation takes place in the behaviour of the strains used. In the musts fermented with strains 59 and 9080, one may note a partial recovery of the enzymic activity that probably passes from a latent state to a free state. Thus, the final fall in the enzyme after racking, associated with the exponential increase of the colour and with the decrease in phenols, is due to the total involvement of the enzyme in the oxidation of phenols. In the musts fermented with strain 633, the tyrosinase activity runs out during the exponential phase of growth and is not detectable at racking. Neither browning nor decrease in phenols are recorded in this case.

It has already been pointed out that in the musts fermented with strain 633 the tyrosinase activity decreases much more rapidly than in the musts fermented with other strains. It has also been reported that this strain produces substantial amounts of $SO_2$. The inhibitory action of $SO_2$ on PPO is well known (PREMUŽIĆ *et al.* 1972, IVANOV 1967, 1969, IVANOV and IVANOVA 1969, DUBERNET and RIBÉREAU-GAYON 1973). For the purpose of finding a possible correlation between the production of $SO_2$ and the action on the enzyme, we have examined a series of strains of *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis,* either producing $SO_2$ or not, and have determined the influence of the wines produced by them on the tyrosinase activity *in vitro*. The enzyme used in these tests has been the tyrosinase from mushroom, as reported in the methods. Results are shown in Table 1. Apart from two cases (strains 59 and 644), the wines produced with all the strains used have been found to reduce considerably the activity of the enzyme.

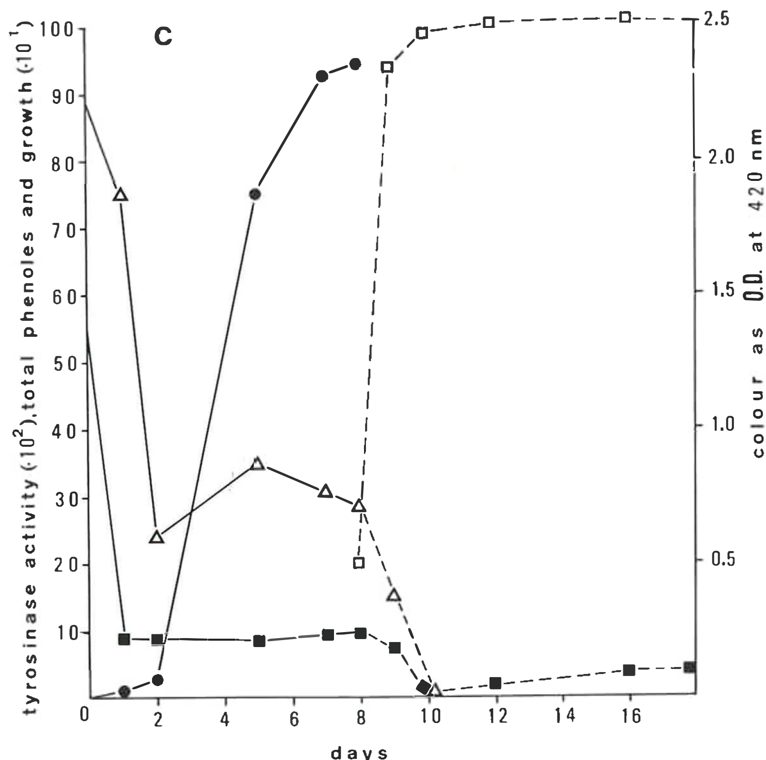


Fig. 1 c: Legend see Fig. 1 a.
Voyez la légende de Fig. 1 a.

The tyrosinase activity is sensitive to the action of pH: In these tests, the wine pH has been buffered by an acetate buffer at pH 4.5, in which the reagents and the enzyme had been dissolved. From the comparative data on the amount of $SO_2$ thus produced, one can note that the wine obtained by strain 633, containing greater amounts of $SO_2$, always exerts an *in vitro* action on PPO, but that there are also samples, containing a very low amount of $SO_2$, that exert a marked inhibitory action on the enzyme (such as 670, 36, 604, 26). In the wines obtained by the above mentioned strains, the inhibition of the tyrosinase activity is not necessarily associated with colour stability, as in the case of strain 640 that inhibits the enzyme by 85% and, in spite of that, gives rise to a brown wine.

The inhibitory power on tyrosinase is therefore fairly widespread in yeast, although, quantitatively, to different extents.

*In vitro* inhibition of the tyrosinase activity

It has already been pointed out that in most cases the inhibition of *in vitro* tyrosinase activity by yeast strains is not associated with the production of $SO_2$. For the purpose of ascertaining whether or not such yeasts were also producing in synthetic media one or more compounds capable of inhibiting the enzymic activity, we have cultured, as described in the methods, nine strains and have tested the influence of 6 days' culture overnatants on the *in vitro* enzymic activity. 1 ml of 6 days' culture liquid has been held in contact with 9 $\mu$g of enzyme dissolved in an

Table 1

Effect on the tyrosinase activity *in vitro* of the wines fermented by various strains of yeast.

Influence sur l'activité de la tyrosinase *in vitro* des vins fermentés par différentes souches de levure.

| Strain | PPO inhibition %[1]) | O.D. at 420 nm[2]) | $SO_2$ produced meq/l |
|---|---|---|---|
| 670 | 90 | 0.14 | 0.1 |
| 604 | 85 | 0.29 | tr.[3]) |
| 640 | 85 | 0.96 | tr. |
| 633 | 85 | 0.13 | 2.1 |
| 26 | 80 | 0.30 | 0.5 |
| 36 | 80 | 0.16 | 0.4 |
| 60 | 70 | 0.63 | 0.2 |
| 632 | 70 | 0.56 | 0.3 |
| 630 | 60 | 0.56 | 0.8 |
| 659 | 60 | 0.86 | tr. |
| 109 | 60 | 0.38 | 0.5 |
| 627 | 50 | 0.57 | 0.0 |
| 610 | 48 | 0.68 | tr. |
| 657 | 30 | 0.59 | tr. |
| 642 | 30 | 1.44 | tr. |
| 645 | 26 | 1.26 | tr. |
| 59 | 00 | 1.49 | 0.4 |
| 644 | 00 | 1.31 | tr. |

[1]) Percentage of inhibition, calculated against the control, after 10 minutes of contact.
[2]) Colour 48 hours after air exposition.
[3]) Trace.
Data represent means of 5 replicates.

acetate buffer solution at pH 4.5 for 0, 2.5, 5, 7.5 and 10 minutes. Then the reaction substrate (5 mg of pyrogallol dissolved in 1 ml of buffer) has been added. Reaction time: 10 minutes. Culture overnatants of strains 633, 640, 604, 670, 26, 36, 58 inhibited the tyrosinase activity, while the behaviour of strains 59 and 9080 has been comparable to that observed in wine experiments. Fig. 2 shows the inhibition percentages relating to the culture overnatants of strains 633, 36 and 27. Also at null contact time, strains 633 and 36 have a marked inhibitory action that increases, approaching 100%, after 10 minutes' contact. Also strain 27 exhibits an inhibitory action that becomes total only after 10 minutes' contact.

### Influence of nutritional conditions

As has already been said, the production of a tyrosinase-activity inhibiting product by the yeasts is a widespread, although quantitatively varying, character. Additional tests have been carried out for the purpose of determining whether it is a primary metabolite (always produced by yeasts) or a secondary metabolite, the production of which is largely affected by environmental and nutritional conditions.

Secondary metabolites do not have a specific function in the economy of the cell and are usually produced during the stationary stage. The production of such compounds is subjected to a number of controls and particularly to the "catabolic repression". This phenomenon consists in the reduction of the rate of synthesis of a certain compound, especially connected to the degradative metabolism, in the

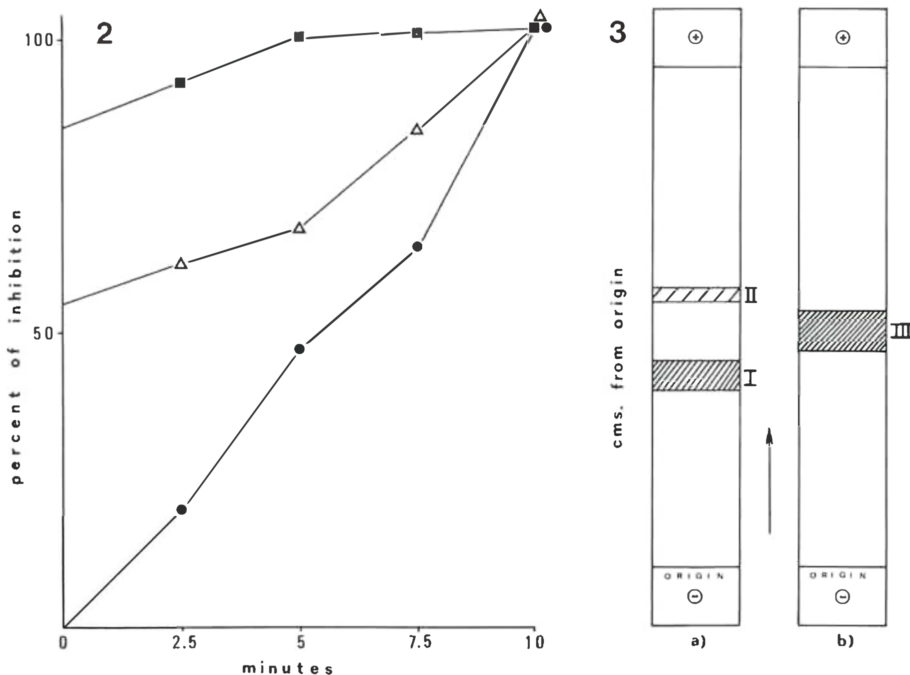


Fig. 2: Effect of contact time on the inhibitory power of the cultural overnatants on tyrosinase activity *in vitro*. Symbols: △ = 633; ● = 27; ■ = 36. Inhibition percentage calculated against the control (1 ml of buffer — 1 ml of enzyme solution — 1 ml of substrate).

Fig. 3: Effect of treatment with culture overnatant of the strain 633 on electrophoretic mobility of tyrosinase from mushrooms: (a) control: protein fractions (I, II) of tyrosinase in 0.3 M borate buffer, pH 8.5; (b) tyrosinase in borate buffer treated for 10 minutes with culture filtrate of strain 633. Electrophoresis, at 2 °C, was done on Whatman 3 MM paper wet with 0.3 M borate buffer, pH 8, and run for 4 hours at 450 volts.

Fig. 2: Influence du temps de traitement sur le pouvoir inhibiteur de l'activité de la tyrosinase *in vitro* des surnageants. Symboles: △ = 633; ● = 27; ■ = 36. Pourcentage d'inhibition calculé par rapport au témoin (1 ml de tampon — 1 ml de solution de l'enzyme — 1 ml de substrat).

Fig. 3: Influence du contact avec le surnageant de la souche 633 sur la migration électrophorétique de la tyrosinase provenant de champignons: (a) témoin: fractions protéiques (I,II) de l'enzyme dans tampon borate 0.3 M, pH 8,5; (b) tyrosinase dans tampon borate traitée avec le surnageant de 633. L'électrophorèse était faite à 2 °C sur papier Whatman 3 MM dans tampon borate 0.3 M, pH 8, pendant 4 heures à 450 volts.

presence of sources of carbon easily utilizable. With a view to determining whether the compound in question is a secondary metabolite subjected to such a control mechanism, we have cultured statically three strains in the presence of various sources of carbon fermented by the yeasts in this preferential order: glucose, saccharose and maltose (GRIFFING 1970). The sources of carbon were present in optimal (5%) and in suboptimal (1%) concentrations. A reduction of the *in vitro* tyrosinase inhibitory activity has been recorded in the culture overnatants of maltose-grown strains. Table 2 shows the data on the inhibition exerted by the 6-day-old culture overnatants of the three strains examined, as a function of the time of contact with the enzyme. It is clear that, from a quantitative standpoint, the production of

Table 2

Effect of carbon source on the inhibitory power of cultural onvernatants on tyrosinase activity *in vitro*. Time of contact between overnatants and the enzyme is varied. Data mean percentage of inhibition calculated against the control.

Influence de la source de carbone sur le pouvoir inhibiteur de surnageants de culture sur l'activité de la tyrosinase *in vitro*. Le temps de contact entre les surnageants et l'enzyme est varié. Les indications signifient le pourcentage de l'inhibition calculé par rapport au témoin.

| Sugar | g/l | Strain 633 | | | Strain 36 | | | Strain 27 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Time of contact (min) | | | | | | | | |
| | | 0 | 5 | 10 | 0 | 5 | 10 | 0 | 5 | 10 |
| Glucose | 50 | 60 | 77 | 100 | 85 | 100 | 100 | 0 | 20 | 100 |
| | 10 | 58 | 75 | 90 | 75 | 85 | 100 | 0 | 15 | 85 |
| Saccharose | 50 | 72 | 100 | 100 | 90 | 100 | 100 | 15 | 20 | 100 |
| | 10 | 59 | 100 | 100 | 60 | 90 | 100 | 7 | 15 | 80 |
| Maltose | 50 | 10 | 50 | 90 | 5 | 10 | 50 | 0 | 2 | 31 |
| | 10 | 20 | 40 | 90 | 10 | 20 | 60 | 0 | 2 | 25 |

the compound depends on the strain and on the source of carbon present. In the presence of maltose, a decrease in production is observed: one cannot therefore speak of "catabolic repression" and consequently of secondary metabolites in that, among the sources of carbon tested, maltose is the one that is less easily assimilable. Other tests on the influence of nutritional factors have been carried out by adding different key metal ions of secondary metabolism such as $Zn^{++}$, $Mn^{++}$, $Fe^{+++}$ in concentrations varying from $10^{-7}$ M to $10^{-3}$ M, but no appreciable variations have been obtained.

## Chromatographic and electrophoretic tests

The 10-times concentrated culture overnatants of strains 633, 59, 9080 and 36 have been chromatographed on Whatman 3 MM paper using the eluents listed in the methods. In order to detect any inhibitory product present in culture overnatants themselves, each chromatogram has been sprayed after run with a solution of enzyme and then with a solution of pyrogallol. By so doing, it has been possible to detect some colourless areas on the yellow background where the reaction had clearly been inhibited. As far as the culture liquids of strains 633 and 36 in particular are concerned, it has been found that they contained a compound having Rf 0.4 with solvent A and Rf 0.62 with solvent B. In a subsequent chromatogram, the area corresponding to the negative reaction has been cut out and eluted with a very small amount of acetate buffer at pH 4.5. Then, the inhibitory action of the elution on the tyrosinase has been confirmed by the usual method.

In order to determine whether the inhibitory compound(s) exert their action by combining directly with the enzyme itself, the enzyme, held in contact with the concentrated culture liquid of strain 633 for 10 minutes, has been subjected to electrophoresis on 3 MM paper; 450 $\mu$g of enzyme and 1 ml of 1 : 10 concentrate culture overnatant of strain 633 have been charged in comparison with the enzyme itself. Interesting results have been obtained: The contact with the culture liquid of the yeast alters the electrophoretic migration of the enzyme, as shown in Fig. 3. The enzyme exhibited two protein bands (cathecholase and cresolase) stained with

amidoblack (Fig. 3 a); these fractions were active on DOPA solution as described in the methods. After incubation of enzyme with overnatant of 633 only, one protein band at 8.5 cms. was observed in the electropherogram stained with amidoblack (Fig. 3 b); this band was inactive on DOPA solution.

It can be inferred, therefore, that the culture liquid of strain 633 contains a compound forming a stable bond with protein, thus preventing the enzymic activity. The above result confirms and may explain the early disappearance of the tyrosinase activity in wine fermentation tests with strain 633.

Action of laccase activity

We have determined whether the inhibitory action of strains 633 and 36 is specific to the tyrosinase activity or can be exerted also on other oxidase enzymes and particularly on laccase from *Botrytis cinerea*. Tests have been carried out by incubating 1 ml of the 5 days' fungus culture overnatant, containing laccase activity, with 1 ml of culture overnatants of strains 633, 107, 59 and 36. After 5 minutes, 1 ml of reaction substrate, potassium ferrocyanide (100 mg in 100 ml), has been added. Incubation time: 2 hours. The chromophore thus obtained has been read at 436 nm. The percentage of inhibition exerted by culture overnatants of yeast has then been calculated with respect to controls (1 ml of buffer + 1 ml of culture overnatant of *Botrytis* + 1 ml of potassium ferrocyanide). The culture liquids of strains 633 and 36, besides inhibiting the tyrosinase activity, inhibit also the laccase activity by 45% and 52% respectively in comparison with the control. On the contrary, the action of strains 170 and 59, exhibiting a 5% and 2% inhibition respectively, appears to be almost inexistent.

## Discussion

The activity of tyrosinase in must and wine depends not only on technological processes but also on the yeast strain used. The tyrosinase activity persists also after the growth of the yeast is completed, sometimes even for months. In other cases, as in the must fermented by strain 633, the enzymic activity disappears completely during the exponential phase of yeast growth. The action exhibited by the above mentioned strain with respect to tyrosinase in wines, is accompanied by its ability to produce, even in synthetic media, one or more compounds binding the enzyme, as shown by the electrophoretic tests as described above. The knowledge of this inhibition requires a more detailed study; on the other hand, the mechanism of action of tyrosinase is complex and not known in detail. Lerner *et al.* (1972) reported that the enzyme undergoes monomer-oligomer transition and that these structures, depending on physico-chemical conditions, exhibit a different activity.

The inhibitory action observed *in vitro*, is also exhibited against the laccase from *Botrytis cinerea*, although we do not know whether the mechanism of inhibition is the same in the latter isoenzymes.

No direct relationship seems to exist between the production of this compound inhibiting tyrosinase and the production of $SO_2$. In fact, whereas in strain 633 the heavy inhibition of the enzyme *in vitro* and in the fermenting must is accompanied by a high production of $SO_2$, in many other strains such inactivation is not associated with an appreciable production of sulphur dioxide.

The colour of wines is the result of a number of complex phenomena which can only partly be ascribed to the tyrosinase activity. As a consequence, the production of a compound reducing the tyrosinase activity is not always associated with colour stability; among the strains acting upon tyrosinase, only strains 633 and 36 give almost always rise to light and stable wines. The results of fermentation tests confirm, however, the role, though a partial one, played by tyrosinase in the browning of

white wines: In wines fermented by strains 633, where the activity is already non-detectable, neither any browning nor any decrease in phenols in the first 72 hours of exposure to air are observed. With other strains, on the contrary, one may note, in the same period, an exponential browning, that is always accompanied by a marked decrease in phenols and by a loss of the enzymic activity. The latter is ascribable to the interaction of the enzyme with the phenolic compounds and with the products of their oxidation. Dawson and Tarpley (1951), Bonner (1957), Loomis and Battaile (1966) and Anderson (1968) report that enzymes, and tyrosinase in particular, are inhibited by the products of oxidation of polyphenols. On the other hand, Traverso-Rueda and Singleton (1973) report that the catecholase activity decreases as a result of the formation of bonds with the phenolic products acting as a reaction substrate; this hypothesis is also reported by Dubernet and Ribéreau-Gayon (1973). According to Loomis (1969), inactivation is due to the oxidation of the fundamental sites of the enzyme by the quinones. This hypothesis may also reasonably explain the concomitance between the fall of the tyrosinase activity and of the fall in phenols during the first 48 hours of growth, followed by a partial recovery of the tyrosinase activity. It is possible that the reactivation may be due to removal of quinoid compounds from the active sites of tyrosinase. In our opinion, the phenomena of oxidation and the modifications in the must, taking place during the first stage of growth, also in relation to the kind of yeast used, have not been sufficiently studied. The fact that, as reported by Dubernet and Ribéreau-Gayon (1973) and White and Ough (1973), repeated prefermentative aerations may affect both the final tyrosinase activity and the colour and stability of wines, underlines the importance of the reactions taking place during the initial stage of fermentation.

## Summary

The course of tyrosinase activity in musts is related to the strain of yeast used; with strain 633 the activity becomes practically non-detectable during the first days of growth. With other strains a drastic drop always occurs during the first 48 hours, followed by a recovery during the exponential phase of growth, in correlation to a substantial fall of phenols and to the exponential rise in colour. The inhibitory power on the tyrosinase *in vitro* is a widespread character in the yeast, even if quantitatively different: this character is not always associated with colour stability. Such a character, possessed to a remarkable measure by strains 633 and 36, is bound to the biosynthesis, even in synthetical media, of a compound capable of becoming bound to the protein units of enzyme, thus preventing the enzyme activity.

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