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Terpenols flavour in the vinification of "Vinhos Verdes"

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INTRODUCTION

What is "Vinho Verde"?

"Vinho Verde" means "Green Wine". We suppose that the term green is closed with the green landscape of the North Littoral Region of Portugal. "Vinho Verde" is a natural product with a medium alcoholic graduation (between 8 and 11.5 degrees), acidulous (fixed acidity higher than 4 g/l in H₂SO₄); it is very fresh, when tasted. The white wines have a citrine colour and the red ones have a rosed or a bright red froth.

Some definitions of wine aroma

The flavour of a wine is the result of the interaction of its chemical components with the senses of taste and smell. Flavour originates from volatile components responsible for the smell and the non volatile ones producing the taste (salty, bitter, sweet and acid). When we talk of flavour in wine, it is now common to distinguish four types (Cordonnier, 1978) related to the biotechnological sequence presented in Fig. 1, which are as follows:

- varietal aroma characteristic of the grape variety, which depends on the cultivar, soil, climate and phytotechnique.

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Fig. 1 - Technological sequence of wine production and its connexion with the different types of aroma

- pre-fermentary aroma from the grapes and due to the changes caused during the processing of the grapes-pressing, maceration, clarification and others.

- fermentary aroma which depends on the method of fermentation (temperature, yeast strain).

- "bouquet" or postfermentary aroma resulting from the transformations occurring during conservation and ageing.

The varietal aroma is the part of a wine flavour related to the cultivar from which it is produced but may not be directly recognizable in the grape. This means that the specific varietal aroma originates during fermentation and/or conservation of the wine, and neutral taste cultivars
can produce aromatic wines with very particular characteristics. This
definition of varietal aroma (Cordonnier, 1978) introduces the concept of
flavour precursor because two different substances have been found in the
grape:

- the 'odorant', typical of a given variety and existing in the grape,
  transferring completely to the wine and transmitting this typical note.

- the 'odorigenous' equally typical of a given variety but whose
  flavour is only revealed after fermentation and conservation.

The terpene alcohols as markers of some varieties.

Bayonove (1971) attributes the flavour of the muscat cultivar to the
presence of small quantities (a few µg/l) of monoterpenic alcohols. Later
other authors have studied and characterized the varietal aroma of
different cultivars concerning this type of compounds, [Ribéreau-Gayon

Terpenic alcohols among which we can mention linalool, citronellol,
erol, geraniol and α-terpineol appear in the grape in a free state and as
terpene heterosides-precursors.

The latter do not participate directly in the wine flavour, because
due to their molecule size they do not impress the nasal mucous membrane
and because they are insipid in the concentrations found in wine (Noble,
1988). The smell perception threshold value of the various terpenols varies
according to the compound, and, the most aromatic are geraniol and
linalool with perception thresholds around 100-130 µg/l.

Both erol and α-terpineol have perception thresholds 3 to 4 times
higher than linalool (between 400 and 500 µg/l); it has also been noted that
terpenols interreact and a mixture of these compounds is more aromatic
than the most aromatic of the components in its pure state (Ribéreau-
Gayon, 1975; Etiévant, 1983; Rapp, 1986).

The distribution of monoterpenic alcohols in the vine and in the berry.

Monoterpenic alcohols (free and precursors) are differently spread
in the vine, mainly on the leaves, the stalk and the berry and its quantity
depends on the maturation state (Cordonnier, 1978; Gunata, 1985 and
Boniface, 1987).

It has been observed that both glycosidically-bound monoterpenes
fraction and the free one increase with maturation, reaching its peak some
time before vintage; this fact suggests the use of free and bound terpenes
concentration as a evaluation criteria for the level of maturation of the
grape (Reynolds, 1989).
Fig. 2 - Chemical structures of terpenic alcohols and glycosides of the musts and the wines.
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Considering only the berry, a predominance of varietal aromas is found mainly in the skin (Cordonnier, 1981), whose distribution differs according to the compound; geraniol and nerol are mainly found in the skin, whereas linalool is divided almost in equal parts between the juice, on the one hand, and the skin and cell fragments on the other. Therefore, the skin maceration is an interesting technology as far as the extraction of these types of aromas are concerned (Dubourdieu & Olivier, 1986; Teste 1986, Baumes, 1988) even the possible formation of undesirable herbaceous aromas (Berta, 1986) and of aminoacids which may originate higher alcohols.

The non-volatile fraction (precursors) has been identified as composed of disaccharide glycosides, where the aglycone part, formed mainly by terpenic alcohols, 2-phenyl-ethyl alcohol and benzyl alcohol (Fig. 2) is connected to a glycidic part, formed by a complex mixture of α-L-rhamnofuranosyl-β-D-glucopyranosides e α-L-arabinofuranosyl-β-D-glucopyranosides (Gunata, 1984).

It is possible to liberate the masked aromatic potential

The works of Gunata (1985) have shown that the bound fraction exists in the berry in higher proportion than the free fraction and that it is possible liberate this "masked" potential by means of hot acid hydrolysis at the juice pH. An enzymatic hydrolysis was also suggested, which is less destructive than acid hydrolysis, and uses enzymatic complexes with β–glucosidase activity, naturally present in the grape.

Following this line, Rapp (1985) as he studied the deacidification of the musts and wines using calcium carbonate found that the concentration of monoterpenic alcohols increased (citronellol, geraniol, nerol and others) due to the β–glucosidase activity of the musts and the wines which caused the enzymatic hydrolysis of terpenic glucosides present.

High concentrations of β–glucosidase were found in grape berry solids (skin and pulp) and low concentrations in the juice; the juice is particularly rich in bound terpenols. This distribution is similar to that of free terpenols in the same parts of the berry and not to the bound terpenols. The β–glucosidase content is very high in vine leaves and low in stems. Enzyme activity increases during the maturation of the fruit. It was found that in mature fruit of both aromatic and nonaromatic cultivars, β–glucosidase activity varied according to the cultivar for a given vintage (Biron, 1988) and was frequently found to vary considerably from one vintage to another.

On the other hand the vine cells can synthetize the terpenic glucosides from the respective alcohols and the metabolic means leading
to the formation of these compounds has been established (Paisanrat, 1985).

Thus the possibility of interconversion between free and precursor forms appears as an interesting technological aspect because it can stress an aromatic potential that remains inodorous using the traditional winemaking processes.

Following this, Cordonnier (1989) presented the results of a comparative study of the various enzymatic preparations on the market, assessing their capacity of hydrolysing the terpenic glucosides extracted from muscat grapes. Nevertheless, although the application of these methods allows the knowledge of the global aromatic potential of a given cultivar, the use of exogenous enzymes during alcoholic fermentation is dangerous because we run the risk of losing the character of the respective wines.

The technology used influences the wine composition

The composition of the must to be fermented is determinant for the characteristics of the final product, reason why the technological processes used have a decisive influence over the wine flavour.

The type of harvest (manual or mechanical), destemming, pressing, maceration, oxigenation and clarification condition the transparency of the musts and the formation of prefermentary aromas that appear in the last stages of fermentation.

The clarification of the musts (racking) is normally indispensable to obtain the finest expression of a varietal aroma. It can be performed by static defecation, centrifugation or vacuum filtration (Dubourdieu 1986). As the colloidal fraction of the must (glycoproteins, polypeptides, polysaccharides) is capable of adsorbing certain inhibitors, it may stimulate the alcoholic fermentation and liberation of some aromas (Houtman & Marais 1980; Houtman & du Plessis, 1985).

During fermentation the aromatic profile is modified due to the terpene compounds

Generally, during alcoholic fermentation a reduction of bound fractions of terpenic components is observed, and the evolution of the free fractions varies considerably, according to the different compounds and must composition. Gunata (1986) verified that during alcoholic fermentation of musts from muscat cultivars, the concentration of free linalool remains practically constant, presenting a decrease of nerol and geraniol levels, in particular the latter; on the other hand α-terpineol increases several times in relation to its initial concentration.
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As the enzymatic equipment of the grape is known, several authors namely Dubourdieu (1988) and Darriet (1988) studied the role of the yeast Saccharomyces cerevisiae in the enzymatic hydrolysis of terpenic heterosides found in grape juice, concluding that this yeast is responsible for the release of small quantities of free terpenols, mainly nerol and geraniol. The quantities released during fermentation compensate for the loss of volatile terpenols in the course of fermentation due to the carbon dioxide effluent (Miller, 1987).

The aromatic profile of the wine depends on the yeast strain

Marchetti (1987) and Cabrera (1988) studied the effect of the interaction yeast strain/must composition on the production of volatile metabolites, concluding that certain strains can have a stronger effect on the composition of the wine than the one derived from the must and also that the flavour composition of the wine obtained depended on the yeast used.

This fact opens a new field to the use of selected yeasts whose implantation in must is often difficult (Razière, 1989) due to the competition with indigenous yeasts. The diversity of strains available on the market allows a qualitative intervention on the chemical and organoleptic characteristics of wine. Nevertheless it is necessary to study in detail the factors that intervene to determine the implantation of a given strain, i.e., growth rate, the demands in nutrition elements, the active inhibitions of some yeasts over others (Strehaiano, 1985), and the manifestations of the killer phenomenon.

The "state of the art" concerning "vinhos verdes"

A considerable part of the varietal flavours research has concentrated mainly on strongly aromatic cultivars from France, Italy, Germany, Australia, California, South Africa. As far as Portuguese cultivars are concerned, there seems to exist no systematic study in this area, with the exception of some work on the characterization of some white cultivars from the Vinho Verde Region, carried out by Andrade (1990), Oliveira (1991) and Guedes de Pinho (1991).

Considering the use of selected yeasts, some isolated tests have been carried out in local cellars which, although still inconclusive, suggests promising results.

The aim of this work

The objective of this work is to characterize the flavour profile of some Vinho Verde Region cultivars and to study the influence of the use of three commercial dry yeast strains on the flavour profile of the wine of the "Loureiro cultivar".
MATERIALS AND METHODS

Juices - sound and mature berries of seven white cultivars were obtained from the vineyards of the Vinho Verde Region. Each berry was separated from the stalk by cutting the pedicle at the base and was crushed and pressed at the atmospheric temperature to obtain the juice. After centrifugation (9000g x 15 min at 4 °C) the juices were deepfrozen at -20 °C and stored at this temperature for subsequent analyses.

Yeast - Three strains of active dry yeasts were used as follows:

- Saccharomyces bayanus, isolated from the indigenous flora in the "Vinho Verde Region" (A yeast).
- Saccharomyces cerevisiae, selected in Montpellier region between yeast strains with killer character (B yeast).
- Saccharomyces cerevisiae, selected by Institut d'Oenologie de Bordeaux (C yeast). Its enzymatic equipment permits the hydrolysis of the precursors of aroma. With neutral character vis-à-vis killer phenomena.

Winemaking - Grapes of Loureiro variety were harvested during the final stage of ripening (official vintage in the Amarre region) and passed in a Voslin press (first and second pressing). Sulfur dioxide solution was added to give an overall concentration of 60 mg/l. The static defecation during 24 hours at 14°C was applied and racking with aeration to 500 l vats. Three actively fermenting yeast cultures at the maximum recommended dosage were inoculated to each vat and fermentations were conducted at 14±2°C.

Bentonite was added during fermentation when the density of the juices was approximately 1050 g/l. Sulfur dioxide is used here with the elimination of yeast by centrifugation. Two litres samples of each vat are collected and stored for analyses.

Extraction of volatiles - A sample of 100 ml of juice or wine was successively extracted with 2 ml, 2 ml, 1 ml of an ether-hexane mixture (or only hexane). One millilitre of a 10 mg/l solution of β-octanol as an internal standard and sodium chloride (salting out) were added before extraction. Three extracts and the rinsing solution were concentrated with nitrogen and kept at -20°C until tested.

Chromatographic determination - Terpenoid compounds and other alcohols were determined using a CPWAX 52 CB (50 m x 0.32 mm) glass capillary column connected to a FID detector with helium as carrier gas. Linalool, α-terpineol, citronellol, nerol, geraniol, benzyl and 2-phenethyl alcohols were identified by comparing their retention times with the corresponding standards by coinjection. The possibility of the enlarge-
Fig. 3. Flavour profiles of some cultivars from Vinhos Verdes Region, concerning the terpenic alcohols and others which constitute the aglycone part of aroma precursors. (* - extraction with hexane).

ment of the time scale offered by the acquisition data system permits to detect very small and adjacent pics.

RESULTS AND DISCUSSION

Fig. 3 represents the flavour profiles of seven cultivars from the Vinhos Verdes Region, namely Loureiro 1990, Loureiro 1991, Trajadura, Pedernã, Azal (Viana do Castelo), Esganinho, Avesso and Verdelho, concerning the alcohols which constitute the aglycone part of aroma precursors, that is, linalool, α-terpineol, nerol, citronellol, geraniol, 2-phenyl-ethyl alcohol and benzyl alcohol.

The grapes of Loureiro variety 1990 were manually crushed and pressed and the ones of Loureiro 1991 were pressed in an industrial press; this fact explains the more aromatic profile of Loureiro 1991 must.

The grapes of Azal cultivar, typical of an inland region, were harvested at Viana do Castelo (littoral region); however the differences in the soil and in the atmospheric conditions can mask the characteristics of this cultivar.

As can be observed, the Loureiro cultivar typical of the Lima Valley Region is the most aromatic (total terpenols = 686 µg/l and total alcohols of the aglycone part = 1296 µg/l.).
The results of the chromatographic analyses are in complete accordance with the organoleptic characteristics known to these cultivars.

The histogram presented in Fig. 4 shows the aromatic profile of wines obtained with three commercial dry active yeast strains-starting with the same Loureiro must, fermented at equal temperature, airing conditions and other conventional procedures.

It was verified that there is no considerable difference in the three wines as far as volatile terpenols are concerned, which was also realized by the "tasting" of young wines. The quantities of terpenols are probably below the perception threshold value.
There is a decrease of the α-terpineol concentration in the wines, due to the carbon dioxide effluent that causes a loss in the course of fermentation. The sole component that increases is 2-phenylethanol, the A yeast being the responsible for the largest increase.

The unexpected differences among the three wines might be due to difficulty of implantation of yeasts selected in other regions namely B yeast (Montpellier) and C (Bordeaux) which compete with wild yeasts. In fact, a microbiological analysis carried out at the end of fermentation with strain B proved that this yeast was not implanted.

Althought this strain possesses the killer factor, the difficulty of implantation may be due to the clarification process used in this case (static decantation) that allowed the stable wild population of the must before inoculation, as observed by Rozière (1989). This hypothesis can only be confirmed by cariotype analysis of the respective yeasts.

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