Wine colour adsorption phenotype: an inheritable quantitative trait loci of yeasts

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Abstract

Aims: In this work, a population of 88 descendants derived from three wine strains of Saccharomyces cerevisiae was tested for the enological trait 'wine colour adsorption' (WCA) to evaluate its inheritability.
Methods and Results: The WCA phenotype was tested on plate agar medium specifically formulated for the purpose. After 10 days of anaerobic incubation at 28°C, a computer-assisted assessment of WCA aptitude of the yeasts was carried out. The biomass colour – ranging from white to dark brown – reflects the adsorption of grape pigments: white and dark brown biomass colour corresponds to low and high adsorption, respectively. In order to confirm biomass colour results, microvinification trials using red must were performed, and the obtained wines were analysed.
Conclusions: The analysis of the progeny demonstrated that the enological trait WCA is inheritable and polygenic.
Significance and Impact of the Study: A way to describe the polygenic effect of the WCA trait has been found, also showing that this trait is inheritable. The impact of the work revolves more around the large-scale screening method, which could then assist in breeding wine yeast, and can also be used as a scientific tool to investigate WCA trait.

Introduction

The selection of micro-organisms, including those that have been genetically improved, is based on the identification of a specific function that can be assessed through phenotypic or metabolic methods. In the case of wine yeasts, it is necessary that the selected strains possess some basic traits together with others, more specific and functional to the type of wine. Wine yeast improvement is a continuous process, meeting the current requests of the winemaking field (Giudici and Zambonelli 1992; Giudici et al. 2005), and therefore, the number of enological traits potentially exploitable is ever more on the increase.
It is well known that yeasts can influence wine colour (Castino 1982; Conterno et al. 1997; Sacchi et al. 2005; Stockley and Høj 2005), either by anthocyanin adsorption on yeast cell walls (Vasserot et al. 1997) or by periplasmic anthocyanin-β-D-glucosidase intervention (Delcroix et al. 1994; Sponholz 1997; Manzanares et al. 2000). Wine colour is also modified by the amount of acetaldehyde and pyruvic acid produced by wine yeasts during alcoholic fermentation (Dallas et al. 1996; Fulcrand et al. 1998; Liu and Pilone 2000; Morata et al. 2003b, 2006; Lopez-Toledano et al. 2004). Furthermore, pectinolytic enzymes also secreted by wine yeasts (Hernández et al. 2003) increase the extraction of colour from pomace (Fernández et al. 2000; Ribéreau-Gayon et al. 2000). The high capacity of flor yeasts to retain coloured compounds explains their protective effect against browning (Merida et al. 2005) and their use in colour correction of white wines (Bonilla et al. 2001; Razmkhab et al. 2002). The colour stability of red wines can be increased by anthocyanin combination with yeast polysaccharides, principally mannoproteins (Ferrari et al. 1997; Escot et al. 2001; Feuillat et al. 2001; Feuillat 2003).
The colour intensity of wine is affected by contact with yeast lees, whereas colour tint is not (Salmon et al. 2002). Effectively, yeast hulls can adsorb both flavans and anthocyanins in polymeric form, showing a great affinity for flavonols (Ummarino et al. 2001); after 1 week of contact in a synthetic model wine, the proanthocyanidins decreased up to 70%, the total tannins by 38.1% and the anthocyanins by 14.7% (Mazauric and Salmon 2005). Some authors are not in agreement with previous data and hypothesize that anthocyanin derivatives are directly correlated to wine colour and not to yeast adsorption (Eglinton et al. 2004). According to other authors, yeast strain slightly influences the total anthocyanin content of wines (Mazza et al. 1999; Bosso et al. 2002; Malacrinò et al. 2005). Anyway, significant or remarkable differences in colour, polyphenolic index and anthocyanins were diffusely reported for wines fermented with different yeast strains (Cuinier 1988; Dumont et al. 1993; Delteil 1995; Boisson et al. 2002; Morata et al. 2003a, 2005; Caridi et al. 2004; Medina et al. 2005).
Based on these data, it is possible to delineate the enological trait 'wine colour adsorption' (WCA). The unavailability of a relevant and reliable test is the bottle neck to quickly screen a large population of yeast strains for technological trait selection. Recently, a very simple method, based on evaluation of the biomass colour of yeast colonies to determine the wine yeast aptitude to adsorb pigments from grape skins and seeds has been developed (Caridi et al. 2002; Caridi and Cufari 2003; Caridi 2005). The assumption was that biomass colour reflects the binding of grape pigments to the biomass.

The aim of this work was to analyse the inheritable nature of the WCA phenotype using the screening method previously cited, and confirming the results by microvinification trials.

Materials and methods

Strains, media and growth conditions

The study was carried out using three parental wine strains of Saccharomyces cerevisiae, named TP5, TT173 and TT254, and 88 single-spore cultures of which 32 were obtained from TP5, 14 from TT173 and 42 from TT254. The parental strains were isolated from native microflora of wine fermentations and selected as regards the most important enological traits (Caridi et al. 1999). The yeasts were grown at 28°C for 2 days on YPD medium (1% yeast extract, 1% peptone, 2% dextrose), solidified with 2% agar when required.

Sporulation and isolation of spores

Sporulation was induced at 28°C for 7 days on acetate medium (1% anhydrous sodium acetate, 2% agar). Ascospores were isolated on YPD agar by a micromanipulator Singer MSM System series 300 manual. Ascus wall was digested at 25°C for 20 min using zymolyase 20T-10 mg ml^{-1} (Seikagaku, Kogyo／Tokyo, Japan) diluted 1 : 9 with sterile distilled water. The germination efficiency was expressed as the percentage of isolated spores forming a colony at 28°C after 3 days.

Determination of the WCA trait on yeast biomass

The WCA phenotype study was carried out using the chromogenic plating medium grape-skin agar (Caridi et al. 2002; Caridi and Cufari 2003; Caridi 2005) buffered at pH 3.5 and modified as follows. Homogenized grape skin (100 g l^{-1}), peptone from casein (18.75 g l^{-1}), yeast extract (11.25 g l^{-1}), glucose (50 g l^{-1}), Na_2HPO_4 (62.5 g l^{-1}), and citric acid monohydrate (125 g l^{-1}) were suspended in distilled water, treated at 100°C for 30 min, filtered through gauze, distributed into test tubes (4 ml per tube) and autoclaved at 121°C for 15 min. A solution of agar (33.33 g l^{-1}) was prepared, distributed into test tubes (6 ml per tube) and autoclaved at 121°C for 15 min. One test tube containing
the medium and one containing the agar solution were poured together into Petri plate (ø 60 mm) and carefully mixed using a sterile L-shaped plastic spreader. After solidification, each plate was used to inoculate one yeast strain, spreading a small quantity of yeast biomass, previously grown at 28°C for 2 days on YPD agar, over the surface of the plate. After 10 days of anaerobic incubation at 28°C, using AnaeroGen gas-pack catalyst (Oxoid LTD, Hampshire, England), a computer-assisted evaluation of biomass colour of the yeast colonies was carried out. The biomass colour, ranging from white to dark brown, reflected the adsorption of grape pigments – white and dark brown biomass colours
corresponded to low and high adsorption, respectively. Yeast biomass was taken using a calibrated loop, carefully mixed, and spread on the loop. The biomass was photographed using the digital camera hp Photosmart 945, and the image was processed for colour. Photoshop CS for Windows XP from Adobe was used to perform red–green–blue (RGB) analysis. The region of interest was set to 5 x 5 pixels, taking four replicates for each strain. Photoshop's RGB colour mode assigned an intensity value to each region. The intensity values ranged from 0 (black) to 255 (white) for each of the RGB components in a colour image. Accordingly, a low WCA aptitude corresponded to higher RGB values, i.e. white biomass colour; a high WCA aptitude corresponded to lower RGB values, i.e. dark brown biomass colour.

Determination of the WCA trait by microvinification trials

In order to confirm biomass colour results, microvinification trials, using red must from Calabrian grapes of Vitis vinifera, were performed. The grapes are given pre-fermentative maceration to extract pigments from skins and seeds. They were destemmed, crushed and cold soaked at 0°C for 3 days, performing a punch down twice per day. The must obtained after pressing (pH 3.50, °brix 23) was divided in aliquots of 100 ml and fermented at 20°C with the 91 wine yeasts inoculated at 5% in triplicate. Carbon dioxide was measured by weight loss to determine the end of fermentation.
Wines were centrifuged at 4500 rev min^{-1} for 5 min, and the absorbance was read at 420, 520 and 620 nm. The colour intensity was given by the sum of the three absorbances; the colour tint was expressed by the ratio of the absorbances at 420 and 520 nm. The total polyphenol content was determined using the Folin–Ciocalteu (FC) index according to Singleton and Rossi (1965).
Both RGB values of yeast biomass and analytical data of wines were subjected to statistical analysis using StatGraphics Centurion XV for Windows XP from StatPoint.

Results

Progeny constitution

About ten tetrads of each parental strain were micro-dissected, and spore viability, as scored by progeny formation, was measured; sporulation degree and ascospore viability are reported in Table 1. The spore number per ascus ranged from 1 to 4, spore germination from 44% to 95%. The data suggest a heterogeneous viability and are in agreement with the literature on the topic (Johnston et al. 2000; Marullo et al. 2004).

Analysis of the enological trait WCA

The WCA phenotype of the parental strains and their progenies was determined by RGB component analysis on yeast biomass and confirmed by microvinification trials. The majority of the descendants exhibited significant ($P < 0.05$) differences from their parental strains (Fig. 1); different distribution patterns of colour components were shown for each parental strain.

Concerning RGB component analysis on yeast biomass (Table 2), strain TT173 exhibited for the majority of the progeny significantly different values compared with the parental strain, while strains TP5 and TT254 showed the least number of descendants with significantly different values.

Regarding wines obtained by microvinification trials (Table 2), the three parental strains showed over 50% of their progenies with significantly different values for all the chemical parameters, except for strain TT173 concerning 420-nm parameter. In detail, strains TP5 and TT254 showed for the great majority of the progeny significantly different 420 nm, 520 nm, intensity and tint values. A similar behaviour was exhibited by strain TT173 as regards 520-nm parameter.

To investigate the descendant distributions referring to their parents for each parameter, the respective position, expressed as a percentage value, of strains TP5, TT173 and TT254 among their progenies are reported in Figs 2 and 3. On the whole, there was a remarkable variability both among parameters and parental strains, but progeny always showed a Gaussian distribution.

It is interesting to note that progeny deriving from the same ascus sometimes occupied neighbouring positions, and/or, more rarely, the descendants were included in the same homogeneous group (next numbers in italic type) according to Student–Newman–Keuls analysis ($P < 0.05$). This was observed for the TP5 progeny regarding: the red component (4A-4B; 10A-10B; 11B-11C); the green component (8B-8C); the blue component (8B-8C); the FC index (4A-4D; 9B-9C); the 420-nm parameter (1C-1D; 4A-4D; 5A-5B; 9B-9D; 10A-10D); the 520 nm (4A-4D; 5A-5B; 8B-8D; 9B-9D); the 620 nm (4A-4D; 5A-5B; 8A-8B; 10B-10D; 11A-11C); the colour intensity (1C-1D; 4A-4D; 5A-5B; 9B-9D; 10A-10D; 11A-11B); the colour tint (1A-1D; 8C-8D; 12A-12C-12D). Similarly, for the TT173 progeny regarding: the red component (3B-3C); the blue component (3B-3C), the FC index (4B-4D); the 420-nm parameter (1C-1D; 6C-6D); the 620-nm parameter (1A-1C); the colour tint (1A-1D). And, lastly, for the TT254 progeny regarding: the red component (2A-2B; 4A-4D; 4B-4C; 6B-6D; 10A-10B); the green component (2A-2B; 3C-3D; 6B-6D; 9B-9D; 10A-10D); the blue component (2A-2D; 3A-3D; 4B-4C; 5A-5D; 6A-6D; 8A-8B; 9B-9D; 10A-10B); the FC index (2B-2C; 3B-3D; 6A-6B; 7A-7C; 7B-7D; 8A-8B; 8C-8D; 9B-9D; 10B-10C); the 420-nm parameter (1C-1D; 2B-2D; 6A-6B; 8A-8B; 9C-9D; 11B-11D); the 520-nm (1A-1C; 2A-2C; 3C-3D; 6B-6D; 7A-7C; 8A-8C; 9A-9B-9D; 11B-11C); the 620-nm (1A-1D; 2A-2C; 3B-3C-3D; 4A-4C; 7A-7C; 8A-8C; 10A-10B); the colour intensity (1A-1C; 2A-2B-2D; 7A-7C; 9A-9B; 11A-11B-11C); the colour tint (6A-6B-6D; 8A-8D; 10A-10B). This phenomenon may be exploited by trying to cross neighbouring progenies in order to fix the closer observed characters, hence strengthening the strain potentialities.

Considering only the 11 complete tetrads (Table 1), the significant differences for biomass colour and wine parameters between descendant pairs from the same ascus were analysed and reported in Table 3. A very low number of pairs were significantly different, as regards the yeast biomass parameters, while a higher number of pairs were significantly different as regards the wine parameters. Furthermore, as regards the biomass parameters, no tetrad exhibited significant differences for all the six pair combinations; on the contrary, concerning the wine parameters, this was observed for the following descendents from strain TT254: ascus no. 1 (parameter FC index, 420and 520 nm), asci no. 2 and no. 11 (parameter colour tint), ascus no. 4 (parameter 420, 520 nm, colour intensity and tint), ascus no. 7 (parameter 420, 620 nm, colour intensity and tint), ascus no. 8 (parameter 520, 620 nm and colour intensity), ascus no. 9 (parameter 620 nm, colour intensity and tint) and ascus no. 10 (parameter 420).

In order to identify any possible correlation between colony colour and enological traits, all the available data on the three parental strains and on their descendents were statistically analysed. There was a large variability in yeast behaviour, and as expectable, there was not a full correlation among the RGB component of biomass colour and the analytical parameters of wines (data not shown). In order to point out the most differing behaviours of yeast strains, two out of the 11 complete tetrads were chosen – tetrads no. 1 and 9 – together with their parent: strain TT254. These tetrads both exhibited wine parameter segregation, but it was correlated to the biomass parameters only for one tetrad (Table 4).

Concerning the characteristics of tetrad no. 1, descendant 1B gave a remarkable (and significant) segregation of the wine parameters, producing a wine with higher phenolic content, colour and colour intensity compared with the parent and to the other tetrad components. This segregation was not at all correlated to the biomass parameters; in fact, the descendant 1B showed RGB component values not significantly different compared with the parent and the other descendant 1A.

Concerning the characteristics of tetrad no. 9, descendant 9C gave a remarkable (and significant) segregation of the wine parameters, producing a wine with opposite characteristics compared with the former, hence lower phenolic content, colour, and colour intensity compared with the parent and the other tetrad components. In this case, the segregation was strictly correlated to the biomass parameters. In fact, the descendant 9C exhibited RGB component values remarkably (and significantly) lower compared with the parent and the other tetrad components (Fig. 1). As low RGB values

correspond to high WCA aptitude, the descendant 9C possesses a high aptitude to adsorb grape pigments during fermentation, thus significantly contributing to the production of a wine with lower colour and phenolic content.

Discussion

The continuous quantitative variation of the studied enological trait within wine yeast populations can in part be tentatively explained by a polygenic determinism; indeed, the most desirable enological traits are quantitatively and continuously distributed phenotypes determined by cumulative contribute of quantitative traits loci (QTL).

The phenotypic segregation of WCA trait shows an amplitude of distribution peculiar for each strain. Moreover, for several parameters, the respective positions of parental strains among the progeny distribution were somewhat different. According to Marullo et al. (2004), the relations of dominance ⁄ recessivity between alleles involved in the control of a particular trait are different from one strain to another. For each considered parameter, both concerning biomass colour and wine analysis, there are a congruous number of descendants significantly different from parents. This may allow the possibility to improve wine yeast as regards the WCA trait.

It could be useful to ascertain if WCA trait may be correlated with the chemical characteristics of wines, the aim being the identification of yeast strains with improved enological traits. The absence of a full correlation is mainly attributable to the fact that, as preliminarily asserted, the colour and phenolic content of wines are influenced by yeast, over than WCA trait, with a variety of factors. These can be present in each strain at a different level and independently on its WCA trait. Consequently, it is possible to find out two strains with strictly similar WCA aptitude, but producing wines with significantly different colour and phenolic content, and the converse (Table 4). Nevertheless, the determination of the WCA trait on yeast biomass represents, at present, the fastest and simplest way to investigate on yeast aptitude to adsorb grape pigments.

In conclusion, the analysis of the progeny carried out with this study demonstrated that the WCA trait of wine yeast is a polygenic inheritable QTL, partially and interdependently correlated to colour and phenolic content of wines. Overall, it appears that the selection of wine starters based on the parietal adsorption activity of yeast can enhance wine quality, allowing the selection of yeast strains characterized by diversified adsorbing activity with the following effects: (i) the protection of colour during red winemaking; (ii) the removal of residual colour during white winemaking; (iii) the protection of phenolic compounds responsible for the antioxidant activity of wine. These findings constitute an initial step for establishing breeding strategies in order to improve wine yeast as regards the WCA trait.

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Table 1 Sporulation degree and ascospore viability of the yeast strains

Strain	Number of tetrads	Viable spore/ascus (ascus number)					Percent of viability	Total viable spores
		4	3	2	1	0		
TP5	12	2	5	4	1	0	67	32
TT173	8	0	1	4	3	0	44	14
TT254	11	9	2	0	0	0	95	42

Table 2 Wine colour adsorption (WCA) phenotype of three wine strains and their progenies studied by red–green–blue (RGB) component analysis on yeast biomass and confirmed by microvinification trials

	TP5				TT173				TT254			
		Progeny				Progeny				Progeny		
Parameters	Parent	Mean	Range	%*	Parent	Mean	Range	%*	Parent	Mean	Range	%*
Red component	79	62	23–94	43·7	61	63	17–102	71·4	79	75	33–100	46·3
Green component	33	29	2–62	34·4	21	30	1–66	78·6	37	36	3–60	24·4
Blue component	27	27	3–61	21·9	19	30	2–65	64·3	32	31	2–54	12·2
FC index	12·2	12·3	11·1–16·5	56·2	13·3	12·9	11·7–13·9	64·3	12·9	13·0	11·1-15·9	58·5
420 nm	1·065	1·073	0·953–1·440	90·6	1·021	1·045	0·991–1·163	28·6	1·143	1·150	1·004–1·457	95·1
520 nm	1·205	1·212	1·062–1·688	96·9	1·173	1·149	1·073–1·212	92·9	1·216	1·288	0·990–1·733	97·6
620 nm	0·237	0·242	0·202–0·337	59·4	0·218	0·209	0·188–0·235	57·1	0·227	0·241	0·194–0·348	75·6
Intensity	2·507	2·527	2·220–3·464	84·4	2·412	2·403	2·261–2·589	71·4	2·587	2·680	2·228–3·518	90·2
Tint	0·884	0·888	0·825–0·932	90·6	0·871	0·909	0·866–1·024	50·0	0·940	0·897	0·825–1·247	95·1

*Percentage of descendants from each parental strain included in a diverse homogeneous group ($P < 0·05$ according to Student–Newman–Keuls analysis).

Table 3 Ascus number of the descendant pairs showing significant differences ($P < 0.05$ according to Student–Newman–Keuls analysis) for biomass colour and wine parameters

	Ascus number*					
Parameters	A/B	A/C	A/D	B/C	B/D	C/D
Red component	**8**;3;4;**11**	**8**;1;4;9;**11**	**8**; **10**;1;4;7;**11**	1;3;7;9	**10**;1;3;7	**10**;7;9
Green component	**8**;3;4	**8**;1;4;7;9	**8**; **10**;4;7	1;3;7;9	**10**;1;3;7	**10**;7;9
Blue component	3	9	**8**;7	1;9	1;3;7	**10**;7;9
Folin–Ciocalteu index	**8**;**10**;1;3;4;7;9	**8**;1;7;8;9	**8**; **10**;1;3;4;7;8; 10;11	**10**;1;3;4;7;8;9;11	**10**;1;4;8; 10	1;4;7;8;9;10;11
420 nm	**8**;**10**;1;2;3;4;7;9;10;11	**8**;**10**;1;2;3;4;7;8;9;10;11	**8**; 1;2;3;4;7;8;9; 10;11	**10**;1;2;3;4;7;8;9;10;11	**8**; **10**;1;4;7;8;9;10	**8**; **10**;1;2;3;4;7;8;10;11
520 nm	**8**;**10**;1;2;3;4;7;8;10;11	**8**;**10**;1;3;4;8;9;10;11	**8**; 1;3;4;7;8;9;10;11	**8**;**10**;1;2;4;7;8;9;10	**10**;1;2;3;4;7;8;9;10;11	**8**; **10**;1;4;7;8;9; 11
620 nm	**10**;1;3;4;7;8;9;11	**8**;3;7;8;9;11	1;3;4;7;8;9;11	**8**;1;4;7;8;9	1;2;4;7;8;9;11	**8**; 1;4;7;8;9;11
Intensity	**8**;**10**;1;3,4;7;8;9	**8**;**10**;2;3;4;7;8;9;10;11	**8**; 1;3;4;7;8;9;10;11	**8**; **10**;1;2;4;7;8;9;10;11	**10**;1;4;7;8;9;10;11	**8**; **10**;1;2;4;7;8;9;10;11
Tonality	**10**;1;2;3;4;7;8;9;11	**8**;1;2;4;7;8;9;10;11	**8**;2;3;4;7;9;10;11	**8**; **10**;2;3;4;7;8;9;10;11	**8**; **10**;1;2;4;7;8;9;10;11	1;2;3;4;7;8;9;10;11

*Bold type for descendents from strain TP5; normal type for descendents from strain TT254.

Table 4 Biomass and wine parameters concerning strain TT254 and its descendant tetrads no. 1 and no. 9

	Biomass parameters			Wine parameters					
Strains	Red	Green	Blue	FC index	420 nm	520 nm	620 nm	Intensity	Tint
TT254	79	37	32	12·9	1·143	1·216	0·227	2·587	0·940
TT254-1A	78	40	35	12·6	1·072	1·158	0·217	2·446	0·926
TT254-1B	77	44	43	15·7	1·443	1·629	0·304	3·376	0·885
TT254-1C	60	27	26	13·0	1·039	1·180	0·219	2·438	0·881
TT254-1D	66	31	28	13·5	1·050	1·126	0·209	2·385	0·933
TT254-9A	77	35	32	13·0	1·264	1·425	0·249	2·938	0·887
TT254-9B	76	32	29	13·3	1·222	1·420	0·271	2·913	0·860
TT254-9C	43	13	15	11·2	1·232	0·993	0·197	2·422	1·241
TT254-9D	76	32	27	13·2	1·232	1·459	0·286	2·977	0·844

Figure 1 Biomass colour of the parental strain TT254 (a) and its progeny from the ascus 9 (b, from left to right: 9A, 9B, 9C, 9D) after 10 days of anaerobic incubation on grape-skin agar. Yeast biomass was spread on a calibrated loop and photographed; the image was processed to perform red–green–blue analysis.

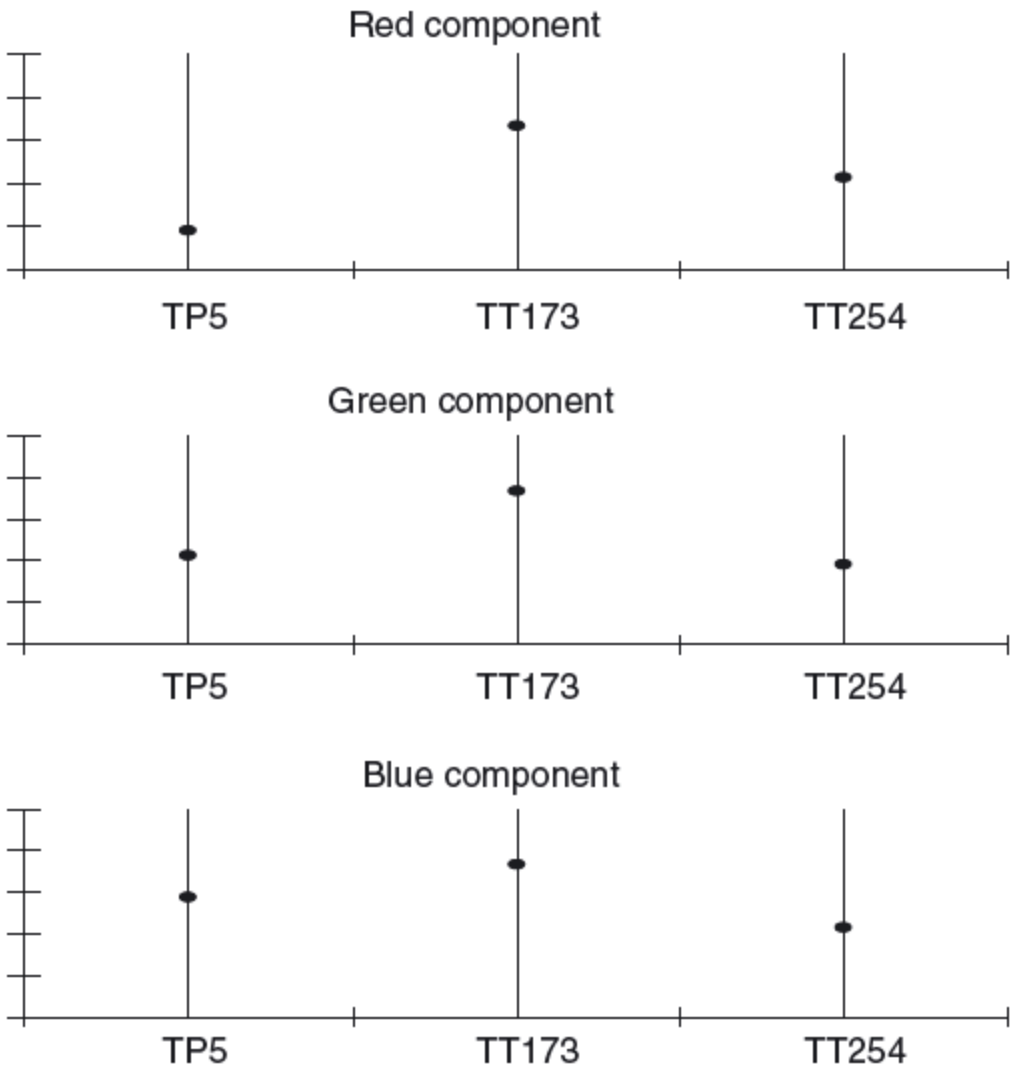

Figure 2 Respective position, expressed as a percentage value, of parental strains among their progenies, considering the red-green-blue (RGB) component analysis on yeast biomass.

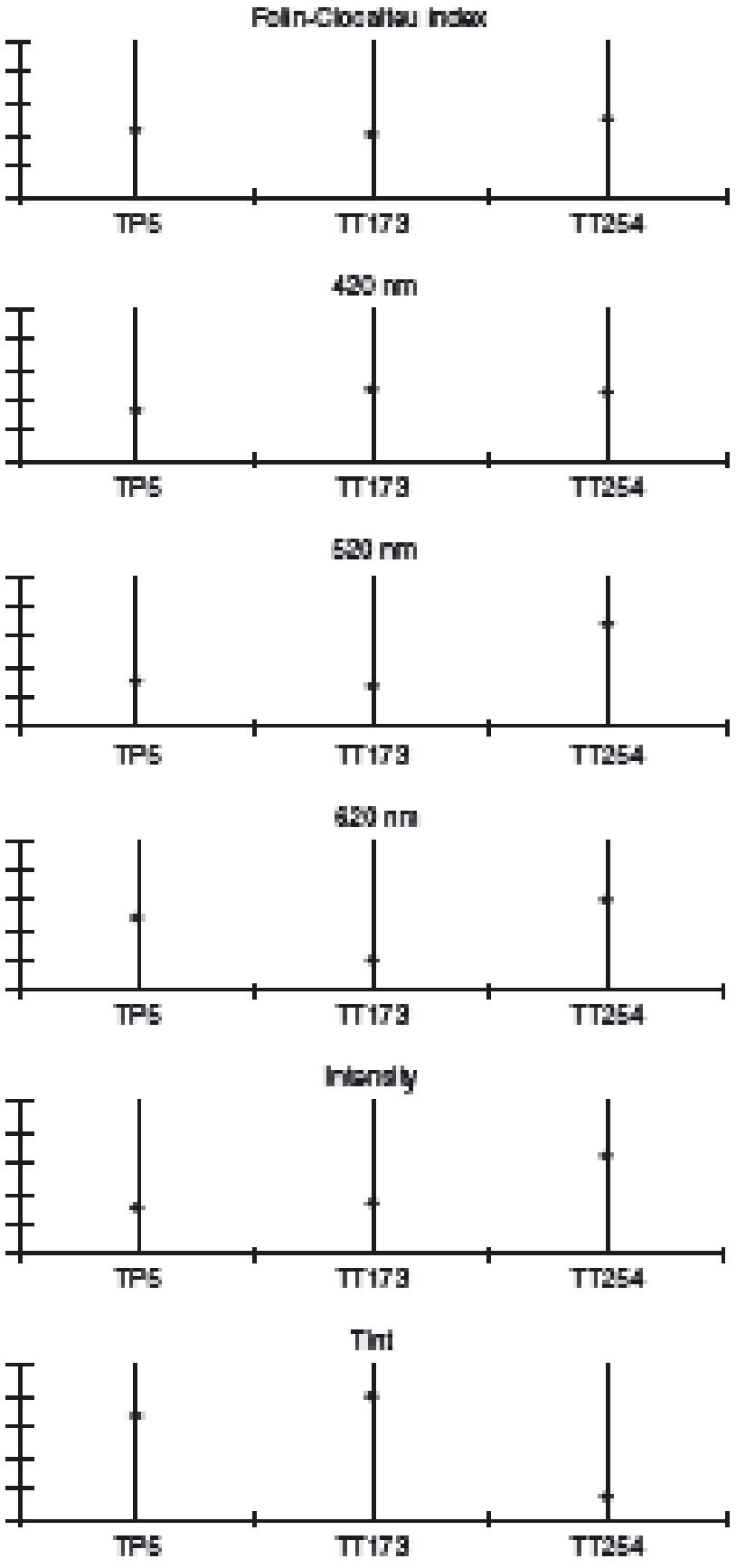

Figure 3 Respective position, expressed as a percentage value, of parental strains among their progenies, considering the chemical parameters of wines obtained by microvinification trials.