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Selected yeasts to enhance phenolic content and quality in red wine from low pigmented grapes

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Abstract

The aim of this work was to enhance - by yeast activity - the quality of red wine produced from black grapes of the Calabrian Gaglioppo variety, used as a model for grapes with reduced synthesis of anthocyanins. Six selected strains of Saccharomyces cerevisiae were used to control winemaking trials. Among the wines there are significant differences, due to the wine starter used. The following technological parameters were significantly different from strain to strain: total acidity, alcoholic degree, tartaric, malic, lactic, and acetic acid, and free and total SO2; moreover, the following phenolic parameters were significantly different from strain to strain: A420, A520, A620, colour intensity, colour hue, Folin-Ciocalteu index, percentage of DPPH inactivation, total anthocyanins, total polyphenols (A280), total tannins, delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside, and malvidin-3-glucoside. From a sensory standpoint, significant differences were observed among wine samples during a short bottle aging. Data validate the main role that wine yeast selection plays in enhancing the quality of red wine from low pigmented grape.

Keywords Low pigmented grapes, Phenolics, Quality enhancing, Selected yeast, Wine

Introduction

Red wine quality is stringently connected to phenolic compounds that contribute to sensorial characteristics and antioxidant effect [1-2]; in addition, red wines with high phenolic content are more attractive, due to their role in reducing the risk of cardiovascular disease and cancer [3-4]. The phenolic content of red wines depends on various factors, such as the climatic conditions of the vineyard and the agronomic techniques [5], the

interactions between polyphenols and other compounds during fermentation [6], the wine yeast activity [7-12], and the winemaking techniques employed [13].

There is a wide genetic variation for berry colour and phenolic content among grape varieties. The genetics and biochemistry of anthocyanin and flavonol biosynthesis is well established in model species; however, the genetic basis of these variations is species-specific and understanding this may be very relevant to the selection of an appropriate yeast strain [14].

Yeast mannoproteins can combine with anthocyanins and tannins in wine; this combination seems to increase colour stability [15] and decrease astringency, giving softer tannins and strongly inhibiting their self-aggregation [16]. Mannoproteins are capable of combining with phenolic compounds, thus diminishing the total polyphenol index; this mechanism may well be exclusively physical, involving the establishment of weak and reversible interactions mainly between anthocyanins and yeast walls by adsorption [17]. Remarkable correlations between the yeast strain used for winemaking and the phenolic composition of wine were shown, highlighting the fact that strain behaviour can somewhat modify the chromatic properties, the phenolic profile and the antioxidant power of wine [8]. At different pH values, the electrical charge of mannoproteins is modified. In the pH range of wine, mannoproteins carry negative charges and, as a consequence, they may establish electrostatic and ionic interactions with the other components of the wine, resulting in the formation of either soluble or insoluble complexes in a process that is strongly dependent on their net electrical charge and on the structure of their functional groups [18-19].

Colour is one of the most important attributes of red wines, and it originates principally from anthocyanins or their derivatives extracted during the winemaking process [20]. Anthocyanins are mainly located in grape skins; their anthocyanin profiles can be used as chemotaxonomy criteria to distinguish grape varieties or even their clones [21-22]. Category, proportion and amount of anthocyanins in black grapes largely depend on grape variety, viticulture practices and weather characteristics [23-25]. In young red wines, free anthocyanins are the principal source of the red colour, though monomeric anthocyanins are not particularly stable and are easily oxidized [26-27]. Most of the red grape cultivars used for winemaking contain variable quantities of anthocyanins linked to acetic, p-coumaric and caffeic acids, known as acylated anthocyanins. The presence of acylated anthocyanins in black grapes used for winemaking affects wine characteristics, and lead to wines with a slightly blue hue [28]. Nevertheless, the absence of acylated anthocyanins in black grapes does not mean that

colour and quality of red wines diminish. It is well known that Pinot Noir grapes, used in Burgundy for making premium red wines, do not contain acylated anthocyanins [29]. This genetic trait is quite rare in black grapes from other areas. Only two red grape cultivars from Southern Italy, Gaglioppo and Tintilia, do not contain acylated anthocyanins [30-31], but their skins are slightly coloured. The major anthocyanin forms in Gaglioppo wines are monoglucosides, as in other cultivars [28]. During the winemaking, and in the early stages of wine aging, the monomeric anthocyanins undergo a wide variety of reactions and new, more stable, anthocyanin-derived pigments are formed by self-association, co-pigmentation, polymerization between flavan-3-ols and proanthocyanidins, and formation of new pigments, such as pyranoanthocyanins and their further polymerized products [26, 32]. This evolution during wine aging contributes to a progressive shift of the red-purple colour of the young red wines to the red-orange colour of the aged red wines. It has been noted that cyanidin is the precursor of other anthocyanidins and it is the most hydroxylated anthocyanin that undergoes oxidation in the early hours after crushing [33]. Anthocyanidins can be tri-substituted (delphinidin-3-glucoside, petunidin-3-glucoside and malvidin-3-glucoside) as in Sangiovese, Barbera, and Pinot Noir, di-substituted (peonidin-3-glucoside and cyanidin-3-glucoside), as in Nebbiolo, Gaglioppo, Sangiovese, and Pinot Noir, or acylated as in Barbera, Dolcetto, and Montepulciano. Tri-substituted and acylated anthocyanins are less influenced by climate and terroir; on the contrary, the di-substituted are more susceptible to the environment and less stable in wines. Bearing in mind the central role of phenolic compounds, there is a need for protocols and wine starters specifically optimized for each grape variety with reduced synthesis of anthocyanins. Thus, the aim of this research was to enhance - by wine starter activity - the quality of red wine produced from black grapes of the Calabrian Gaglioppo variety, used as a model for grapes with reduced synthesis of anthocyanins.

Materials and methods

Grape Variety

Black grapes of the Calabrian Gaglioppo variety from vineyard of Caparra & Siciliani farm, located in Cirò Marina (Crotone, Italy), were used.

Wine Starters

In a previous study, Caridi et al. [34] examined the inheritability and the segregation of the main enological traits related to the adsorption activity; the traits were studied in 65 wine yeasts of the species Saccharomyces cerevisiae - 3 wild types, 24 single-spore descendants, 4 hybrids obtained by crossing the descendants and 34 single-spore descendants derived from the hybrids. Five out the 34 single-spore descendants derived from the hybrids were chosen for the present research due to their different adsorption activity. Thus, a total of six strains of Saccharomyces cerevisiae were used: five Calabrian strains - RC029-2CxRC039-3C(7)-1C, RC029-2CxRC039-3C(7)-3A, RC029-1DxRC039-3C(4)-1A, RC029-1DxRC039-3C(4)-1C, and RC026-3CxRC039-3C(9)-2B - and the commercial strain Zymaflore F15 by Laffort Oenologie (France) as a control strain. The five strains were obtained using the micromanipulator MSM System Series 400 (Singer Instrument Co Ltd, England) from three hybrids of S. cerevisiae - RC029-2CxRC039-3C(7), RC029-1DxRC039-3C(4), and RC026-3CxRC039-3C(9) - descending from three wild types: RC026, RC029, and RC039. These wild types, also belonging to the yeast collection of our laboratory, had previously been isolated from autochthonous microflora of Calabrian wine fermentations and selected for the most important enological traits.

Precultures

Black grapes of the Gaglioppo variety were destemmed, crushed, cold soaked at 0 °C for 3 days, and punched down twice per day. The must obtained after pressing (20 °brix) was adjusted to pH 3.50, divided into six aliquots of 5 mL, 100 mL, 1 L, 5 L (six aliquots of each) and heated at 110 °C for 5 min. The yeast strains were inoculated in the six aliquots of 5 mL of must and incubated at 25 °C. Every 2 days, each preculture was used to inoculate the superior aliquot. The day before the final inoculation, potassium metabisulphite was added to achieve a final SO2 concentration of 100 ppm.

Mesowinemaking trials and Analytical Methods

Six homogeneous aliquots of 60 kg of grapes of the Gaglioppo cultivar - from the same vineyard row, carefully chosen for berry ripeness, location on the vine, shading and state of health - were destemmed and crushed by a mechanical crusher-destemmer. After the addition of potassium metabisulphite, to achieve a final SO2 concentration in must of 100 ppm , the six aliquots were put into six stainless steel tanks, specifically manufactured to perform winemaking. Each tank was then inoculated with 6 L of a 2-day preculture of the

yeast strains and numbered as follows: (1) Zymaflore F15, (2) RC029-2CxRC039-3C(7)-1C, (3) RC029-2CxRC039-3C(7)-3A, (4) RC029-1DxRC039-3C(4)-1A, (5) RC029-1DxRC039-3C(4)-1C, and (6) RC026-3CxRC039-3C(9)-2B. The grape must was analysed, using standard methods, for °brix, pH and total acidity expressed as g/L of tartaric acid [35-36]. The winemaking was monitored daily for °brix, A420, A520, and A620, [37], total monomeric anthocyanins [38], total polyphenols (A280) [39] and for radical scavenging using 2,2-diphenyl-1-picrylhydrazyl (DPPH·) and expressing the result as percentage of inactivation, according to Molyneux [40]. The cap of crushed grapes on the surface of each tank was punched down twice per day. The grape pressing was performed after 5 days for tanks 1 and 2, after 6 days for the others as indicated by the monitoring of the winemaking; a hydraulic wine press Tico 40 produced by Enotecnica Pillan (Camisano Vicentino, VI, Italy) was used, to a pressure of 100 bars, following the standard working conditions suggested by the producer. At the end of fermentation, the nitrogen was gently blown into the tanks for 5 min and then the wines were bottled. The wines were analysed at two, five, eight and twelve months after the bottling for the following: pH and total acidity; alcoholic degree; A420, A520, A620, colour intensity and hue [37]; Folin-Ciocalteu index [35]; total anthocyanins [38]; free and total SO2 [41]; total polyphenols (A280) [39] and total antioxidant activity [40]. According to Weinges and Nader [42] the total tannin content was determined by mean of the property of monomers and condensed 3,4-flavan-diols to oxidize and give coloured anthocyanidins in an acid-alcoholic medium. The analysis of organic acids was performed using a Knauer HPLC system (Smartline Pump 1000) equipped with Knauer Smartline UV detector 2550 set at 210 nm, and a C18 Knauer Eurospher 100-5 (4.6 x150 mm, 5 μm) column fitted with a guard column was used. The solvent flow rate was 0.5 ml min-1 and the analysis was performed at room temperature with an injection volume of 10 μl. Separation was achieved by isocratic condition using an aqueous solution of 0.1% H3PO4 (pH 2.1). Pure standards of tartaric, malic, lactic and acetic acid were purchased from Fluka (Sigma-Aldrich, Milan, Italy). All values were expressed as g/L. The anthocyanins were determined using the analytical methods recommended by the OIV [38]; a Knauer HPLC system (Smartline Pump 1000) equipped with Knauer Smartline UV detector 2550 set at 518 nm and a C18 Knauer Eurospher II 100-5 (4.6 x 250 mm, 5 μm) column fitted with a guard column were used. The identified compounds were then quantified by calibration with the following standards: delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside and malvidin-3-glucoside (Extrasynthese, Genay, France). All values were expressed as % on the total identified anthocyanins.

Sensory analysis

The principle of Quantitative Descriptive Analysis (QDA) is based on the ability to train judges to measure specific attributes of a product in a reproducible manner to yield a comprehensive quantitative product description useful for statistical analyses [43]. The sensory analysis of wines was evaluated on the visual, olfactory and taste attributes. The sensory evaluation of wine samples was carried out by a panel of eight judges between 28 and 50 years of age (four male and four females), who were familiar with the sensory characteristics of various types of wine. The wines were evaluated in individual temperature-controlled tasting booths, and judges were asked to take unsalted crackers and rinse their mouths with mineral water to minimize sensory carryover. 20 mL wine aliquots were served at 20 ± 2° C in wine-tasting glasses, using a completely randomised order. The evaluations were carried out in individual booths under incandescent white illumination. An unstructured linear 10-cm scale (where 0 means "absence of sensation" and 10 means "extremely high sensation'') was used to evaluate nine sensory attributes (Intensity, Ruby red, Frankness, Odour intensity, Fruity, Floral, Robustness, Astringency, Aromatic persistence), as proposed by Stone et al. [44]. The wines were evaluated after 5, 8 and 12 months from bottling. For each session, a different wine bottle was opened. The obtained data were statistically elaborated using analysis of variance and multivariate statistical techniques and then represented graphically.

Statistical Analysis

All the analyses were performed in triplicate; data were subjected to One-way analysis of variance (ANOVA), using StatGraphics Centurion XVI for Windows XP (StatPoint Technologies, Inc., Warrenton, VA, USA). For each value, Fisher's LSD (least significant difference) intervals were scaled in, declaring their significant differences ($P < 0.05$).

Results and discussion

Tables 1-4 report mean values, standard deviations, and homogeneous groups ($P < 0.05$) of analytical parameters determined 2, 5, 8 and 12 months after the bottling. With the only exception of the pH values at the first monitoring time, all the chemical parameters varied significantly ($P < 0.05$) among wines produced by the

different yeast strains at the different times. Regarding the A520 value (red component), the wines were significantly different and the wine produced by strain 5 exhibited the highest value to 8 months from bottling; this was in accordance with the values of total anthocyanins, total polyphenols (A280), Folin-Ciocalteu index, and colour intensity measured in wines obtained using this yeast. After 12 months from bottling, the wine produced by strain 6 showed the most intense red colour (3.479). Regarding the A620 value (blue component) and the colour intensity, the wines exhibited significant differences and were distributed into homogeneous groups in all the observations. The colour hue parameter is related to the type of pigments present in the wine but also to the oxidation degree of the phenolic compounds. After 2 and 5 months from the bottling, the wine produced by strain 4 exhibited the highest value, consistent with the lower value of the anthocyanins, and therefore it is considered a wine that tends to age more rapidly than the others. On the contrary, the wines produced by strain 1 and 5 exhibited after different times of bottling lower colour hue than the other samples, consistent with the total anthocyanin content, and therefore at some times their colour characteristics were better maintained. Regarding the Folin-Ciocalteu index, the wines were distributed into homogeneous groups at the different observations since the bottling and the total phenolic content measured by the Folin-Ciocalteu index confirmed the results of the total polyphenols (A280). The antioxidant activity of wines, expressed as a percentage of DPPH inactivation, showed significant differences among samples. Except the monitoring after 2 months, the highest antioxidant power was observed in wines produced by the Calabrian strains, whereas the wine produced by the control strain showed a lower percentage of DPPH inactivation. Organic acid contents in wines affect their stability, colour and flavour. Regarding the tartaric acid content, this parameter differed significantly depending on the yeast strain used: the wine produced by strain 5 showed the highest amount of tartaric acid respect to the other strains after two and twelve months from the bottling (5.029 and 3.752 g/L, respectively). This result could be ascribed to its ability to limit the acid's precipitation; on the other hand, the wine produced by strain 4 showed the lowest content of tartaric acid, probably for a more accentuated tartrate precipitation (3.709 and 2.758 g/L, respectively). Malolactic fermentation was not clearly developed in the wines after two months from the bottling; successively it proceeded with an expected decrease of malic acid and an increase of lactic acid, in particular in wine produced by the commercial strain 1 after 12 months (1.465 g/L). All the wines bottled from 2 months exhibited levels of acetic acid higher than is usual; this is probably due to the cap on the must surface that remained outside the fluid mass except for the duration of the daily

punch down. However, 12 months after the bottling all the wines did not pass the legal limit of 1.2 g/L of acetic acid, which identifies one of wine faults [45]. Concerning the total tannin content of wines bottled from 2 months, all the values were in general quite high, considering the Gaglioppo variety. The highest level remained after 12 months in wine produced by strain 2 (4.510 g/L) and strain 6 (4.480 g/L), while the wine produced by strain 1 showed the lowest content (3.510 g/L). Looking at the pattern of anthocyanins by HPLC analysis, cyanidin-3-O-monoglucoside is the dominant anthocyanin for all the strains during grape must maceration; it is interesting to note that it is the anthocyanin present at the lowest concentration in many other cultivars. The following anthocyanin profile is characteristic of the Gaglioppo cultivar: high concentration of cyanidin-3-glucoside, followed by peonidin-3-glucoside, malvidin-3-glucoside, delphinidin-3-glucoside, and petunidin-3-glucoside; acylated anthocyanins are absent. Such anthocyanins decrease significantly with aging, with a concomitant increase in condensed products [46]. In fact, most of free anthocyanins will combine or condense with other phenolic compounds in red wines to form more complex and stable pigments, while a relatively small fraction disappears by degradation, oxidation, precipitation, or formation of other colourless compounds, such as castavinols which can act as a reserve of anthocyanins [47]. The evolution of the anthocyanins leads to interesting changes compared to their content before and after pressing (Fig. 1 and 2, respectively); indeed, two months from the bottling, we can observe percentage content of malvidin-3-glucoside and cyanidin-3-glucoside respectively increased and decreased compared to their content before and after pressing (initial data not reported).

The phenolic profile of the wines suggests that the wine starters used are able to extract the greatest amount of anthocyanins and phenolic compounds from the skins and the lowest amount of tannins from the seeds, as previously studied by Caridi et al. [8] on the Gaglioppo grape variety. Although tannins promote the formation of stable red pigments, and would be useful in the early stages of winemaking due to their high reactivity, high tannin content may affect the sensorial characteristics in a negative way. During the bottle aging, the single anthocyanin content in wines decreased in general, with significant differences among wine samples. Depending on stability of compounds, a decrease or an increase in the percentage were observed. After 12 months from the bottling, the peonidin-3-glucoside was present with the highest percentages in wines produced by strain 4, 5 and 6, whereas the delphinidin-3-glucoside and the petunidin-3-glucoside disappeared in all wine samples because less stable.

In Fig. 3 the mean scores obtained by the sensory analysis of the wines after 8 and 12 months from the bottling are reported. Significant differences ($P<0.01$) were observed by judges for the floral and robustness attributes in wines after twelve months of bottling (Fig. 4 B). The wines are distributed in three homogeneous groups for floral attributes, showing the highest scores in the wines produced by strain 1 and 3. The visual attributes of intensity and ruby red were statistically different among samples after eight months of bottling, with the highest scores for wines produced by strain 3 and 4 respectively. The same samples are characterized by the highest fruity and odour intensity attribute scores (Fig. 3 A). Concerning the taste attributes, there were not significant ($p>0.05$) differences in the astringency and aromatic persistence perceptions of the wines. Statistical differences were observed in the wines after 12 months for robustness attribute. During the bottle aging, the judges observed an increase of the aromatic persistence attribute for all the evaluated wines. In particular, this sensory parameter was correlated to odour intensity by Pearson coefficient ($p=0.54$) in the wines after 12 months.

Conclusions

In general, among the six wines there are significant differences, due to the wine starter used, for all the tested parameters. In details, the following technological parameters were significantly different from strain to strain: total acidity, alcoholic degree, tartaric, malic, lactic, and acetic acid, and free and total SO2, Moreover, the following phenolic parameters were significantly different from strain to strain: A420, A520, A620, colour intensity, colour hue, Folin-Ciocalteu index, percentage of DPPH inactivation, total anthocyanins, total polyphenols (A280), total tannins, delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside, and malvidin-3-glucoside. Some of the evaluated Calabrian strains contributed to the colour of wines during bottle aging with values of intensity higher than ones observed in wine produced by commercial strain. In particular, the strain 5 manifested the best aptitude to be used in the performed winemaking of Gaglioppo for the highest total amounts of anthocyanins, polyphenols and for the most intense colour of the wine. The obtained results could be so considered also for a production of new wines with high content of polyphenols. This validates the main role that wine yeast selection plays in enhancing the quality of red wine from low pigmented grape variety.

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Compliance with ethical standards

Conflict of interest: None.

Compliance with ethics requirements: This article does not contain any studies with human or animal subjects.

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Table 1 Chemical composition of the wines 2 months from the bottling

Analytic parameters	Strains*					
	1	2	3	4	5	6
pH	3.29 ± 0.23a	3.29 ± 0.25a	3.42 ± 0.02a	3.50 ± 0.05a	3.30 ± 0.18a	3.45 ± 0.07a
Total acidity	7.24 ± 0.35a	7.73 ± 0.07bc	7.61 ± 0.07b	8.01 ± 0.18 cd	8.23 ± 0.04d	8.63 ± 0.28e
Alcoholic degree	12.00 ± 0.07a	12.50 ± 0.00b	12.75 ± 0.07c	13.10 ± 0.00d	13.30 ± 0.14e	12.75 ± 0.07c
A420	2.530 ± 0.009a	2.530 ± 0.005a	2.541 ± 0.006a	2.779 ± 0.015b	2.945 ± 0.017c	2.776 ± 0.020b
A520	3.373 ± 0.017c	3.276 ± 0.017a	3.341 ± 0.010b	3.456 ± 0.006d	3.970 ± 0.011f	3.693 ± 0.012e
A620	0.773 ± 0.009c	0.701 ± 0.007a	0.718 ± 0.001b	0.867 ± 0.005f	0.832 ± 0.005e	0.820 ± 0.003d
Colour intensity	6.676 ± 0.034c	6.507 ± 0.024a	6.599 ± 0.008b	7.102 ± 0.019d	7.747 ± 0.032f	7.289 ± 0.034e
Colour hue	0.750 ± 0.002b	0.772 ± 0.004d	0.761 ± 0.004c	0.804 ± 0.004e	0.742 ± 0.002a	0.752 ± 0.003b
Folin–Ciocalteu index	62.75 ± 0.87a	70.08 ± 0.63b	73.08 ± 0.38c	69.67 ± 0.52b	78.08 ± 0.76d	77.33 ± 1.01d
DPPH (% of inactivation)	43.23 ± 1.43c	48.99 ± 1.86d	42.92 ± 0.64bc	39.59 ± 2.67abc	38.95 ± 1.41ab	38.78 ± 0.95a
Tartaric acid (g/L)	4.370 ± 0.059b	4.282 ± 0.030b	4.323 ± 0.180b	3.709 ± 0.465a	5.029 ± 0.031c	4.087 ± 0.046ab
Malic acid (g/L)	1.161 ± 0.067b	0.909 ± 0.120ab	0.837 ± 0.054a	1.136 ± 0.198b	0.984 ± 0.012ab	1.173 ± 0.103b
Lactic acid (g/L)	0.121 ± 0.005c	0.033 ± 0.008a	0.020 ± 0.001a	0.045 ± 0.021ab	0.083 ± 0.032bc	0.058 ± 0.020ab
Acetic acid (g/L)	0.447 ± 0.001a	0.534 ± 0.016c	0.380 ± 0.059a	0.614 ± 0.027d	0.527 ± 0.015c	0.586 ± 0.033d
Total anthocyanins (mg/L)	25.23 ± 1.14b	25.69 ± 1.51b	25.56 ± 0.57b	22.79 ± 1.11a	30.60 ± 0.95c	25.27 ± 2.00b
Free SO2 (mg/L)	32.0 ± 0.0ab	29.9 ± 3.7a	34.1 ± 3.7ab	27.7 ± 3.7a	40.5 ± 9.8b	36.3 ± 7.4ab
Total SO2 (mg/L)	61.9 ± 3.7a	61.9 ± 3.7a	70.4 ± 6.4a	64.0 ± 11.1a	87.5 ± 13.3b	72.5 ± 9.8ab
Total polyphenols (A280)	56.6 ± 0,2a	63.2 ± 1.3b	65.5 ± 0.3c	67.2 ± 0.0d	72.4 ± 0.1f	68.5 ± 0.2e
Total tannins (g/L)	5.873 ± 0.008d	9.038 ± 0.019f	5.567 ± 0.011b	7.795 ± 0.031e	5.624 ± 0.004c	5.435 ± 0.017a
Delphinidin-3-glucoside (%)	8.27 ± 0.24b	8.02 ± 0.07bc	7.94 ± 0.01b	7.11 ± 0.30a	7.17 ± 0.12a	13.12 ± 0.30d
Cyanidin-3-glucoside (%)	17.95 ± 1.49c	16.67 ± 0.41c	16.38 ± 0.29c	12.65 ± 0.06b	19.30 ± 0.13e	9.89 ± 0.49a
Petunidin-3-glucoside (%)	10.96 ± 0.29b	10.57 ± 0.17b	10.92 ± 0.24c	17.99 ± 0.34d	10.08 ± 0.06a	18.88 ± 0.04e
Peonidin-3-glucoside (%)	23.81 ± 0.04c	25.27 ± 0.50c	23.90 ± 0.12b	28.31 ± 1.22d	25.35 ± 0.40c	17.80 ± 0.07a
Malvidin-3-glucoside (%)	38.99 ± 0.91c	39.47 ± 0.34cd	40.87 ± 0.06e	33.95 ± 1.31a	38.09 ± 0.71b	40.31 ± 0.16de

Results are expressed as mean values, ($n = 3$) ± standard deviations; identical letters in row indicate no significant differences at $p < 0.05$

* 1 = Zymaflore F15, 2 = RC029-2CxRC039-3C(7)-1C, 3 = RC029-2CxRC039-3C(7)-3A, 4 = RC029-1DxRC039-3C(4)-1A, 5 = RC029-1DxRC039-3C(4)-1C, 6 = RC026-3CxRC039-3C(9)-2B

Table 2 Chemical composition of the wines 5 months from the bottling

Analytic parameters	Strains*					
	1	2	3	4	5	6
pH	3.42 ± 0.02d	3.40 ± 0.01cd	3.36 ± 0.01b	3.42 ± 0.02d	3.33 ± 0.00a	3.39 ± 0.01c
Total acidity	7.14 ± 0.06a	7.83 ± 0.09b	7.70 ± 0.03b	8.38 ± 0.09c	8.32 ± 0.11c	8.63 ± 0.02d
A_{420}	2.610 ± 0.008b	2.581 ± 0.009a	2.573 ± 0.009a	3.305 ± 0.009e	3.016 ± 0.006d	2.983 ± 0.003c
A_{520}	2.849 ± 0.009b	2.728 ± 0.010a	2.734 ± 0.006a	3.250 ± 0.002d	3.265 ± 0.005e	3.165 ± 0.009c
A_{620}	0.883 ± 0.007b	0.803 ± 0.007a	0.807 ± 0.005a	1.121 ± 0.001d	0.942 ± 0.004c	0.945 ± 0.009c
Colour intensity	6.342 ± 0.024b	6.112 ± 0.026a	6.114 ± 0.020a	7.676 ± 0.010e	7.223 ± 0.015d	7.093 ± 0.003c
Colour hue	0.916 ± 0.000a	0.946 ± 0.000d	0.941 ± 0.001c	1.017 ± 0.002e	0.924 ± 0.000b	0.943 ± 0.002c
Folin–Ciocalteu index	43.93 ± 0.61a	49.76 ± 0.45b	51.96 ± 0.27 cd	48.98 ± 0.33b	53.52 ± 0.47d	50.76 ± 0.26bc
DPPH (% of inactivation)	46.70 ± 0.92a	54.89 ± 1.99c	52.55 ± 1.50bc	58.95 ± 1.47d	54.79 ± 2.06bc	52.14 ± 0.91b
Tartaric acid (g/L)	3.069 ± 0.132ab	2.998 ± 0.103a	3.560 ± 0.011d	2.965 ± 0.016a	3.322 ± 0.216c	3.191 ± 0.073bc
Malic acid (g/L)	0.638 ± 0.066b	0.531 ± 0.044a	0.525 ± 0.026a	0.722 ± 0.092c	0.622 ± 0.084b	0.671 ± 0.036bc
Lactic acid (g/L)	0.169 ± 0.023c	0.109 ± 0.019b	0.082 ± 0.006a	0.093 ± 0.003ab	0.087 ± 0.002ab	0.103 ± 0.031ab
Acetic acid (g/L)	0.348 ± 0.150bc	0.276 ± 0.007ab	0.249 ± 0.008a	0.367 ± 0.013c	0.249 ± 0.001a	0.353 ± 0.004bc
Total anthocyanins (mg/L)	25.20 ± 2.61bc	25.92 ± 0.84bc	23.29 ± 2.52b	19.04 ± 2.42a	27.95 ± 0.84c	23.87 ± 1.41b
Total polyphenols (A_{280})	63.5 ± 2.2a	62.6 ± 1.0a	68.3 ± 0.9b	69.6 ± 2.9bc	74.8 ± 2.1d	72.5 ± 2.8 cd
Total tannins (g/L)	4.446 ± 0.170b	4.130 ± 0.120a	4.982 ± 0.090c	4.964 ± 0.120c	4.968 ± 0.300c	5.471 ± 0.140d
Delphinidin-3-glucoside (%)	5.76 ± 0.58b	4.24 ± 0.59a	5.21 ± 0.98b	3.99 ± 0.12a	5.35 ± 0.13b	5.30 ± 0.03b
Cyanidin-3-glucoside (%)	18.94 ± 1.59d	17.18 ± 0.06c	15.82 ± 0.24b	10.87 ± 0.21a	18.23 ± 0.14d	20.24 ± 1.14e
Petunidin-3-glucoside (%)	13.85 ± 0.07b	10.05 ± 0.44a	9.67 ± 0.54a	17.50 ± 0.46c	13.40 ± 0.38b	17.58 ± 0.80c
Peonidin-3-glucoside (%)	43.32 ± 0.73b	49.68 ± 1.77d	45.44 ± 1.12c	30.30 ± 1.35a	49.89 ± 0.14d	46.81 ± 0.94c
Malvidin-3-glucoside (%)	18.13 ± 2.96c	18.84 ± 1.99c	23.85 ± 0.64d	37.33 ± 1.90e	13.13 ± 0.22b	10.06 ± 0.57a

Results are expressed as mean values, ($n = 3$) ± standard deviations; identical letters in row indicate no significant differences at $p < 0.05$

* 1 = Zymaflore F15, 2 = RC029-2CxRC039-3C(7)-1C, 3 = RC029-2CxRC039-3C(7)-3A, 4 = RC029-1DxRC039-3C(4)-1A, 5 = RC029-1DxRC039-3C(4)-1C, 6 = RC026-3CxRC039-3C(9)-2B

Table 3 Chemical composition of the wines 8 months from the bottling

Analytic parameters	Strains*					
	1	2	3	4	5	6
pH	3.28 ± 0.02c	3.25 ± 0.00ab	3.27 ± 0.03bc	3.31 ± 0.01d	3.24 ± 0.01a	3.23 ± 0.01a
Total acidity	6.94 ± 0.04a	7.71 ± 0.04b	7.71 ± 0.17b	8.05 ± 0.04c	8.21 ± 0.04d	8.42 ± 0.06e
A_{420}	3.822 ± 0.259c	3.343 ± 0.058b	3.293 ± 0.136b	3.135 ± 0.113ab	2.908 ± 0.069a	2.987 ± 0.030a
A_{520}	2.881 ± 0.018b	2.765 ± 0.015a	2.843 ± 0.022b	3.019 ± 0.052c	3.173 ± 0.051d	3.024 ± 0.016c
A_{620}	0.700 ± 0.002c	0.629 ± 0.002a	0.653 ± 0.001b	0.848 ± 0.005e	0.735 ± 0.000d	0.735 ± 0.005d
Colour intensity	7.403 ± 0.279c	6.738 ± 0.056a	6.789 ± 0.113ab	7.002 ± 0.125b	6.816 ± 0.025ab	6.746 ± 0.041a
Colour hue	1.326 ± 0.082d	1.209 ± 0.024c	1.159 ± 0.057c	1.039 ± 0.043b	0.917 ± 0.036a	0.988 ± 0.008ab
Folin–Ciocalteu index	27.27 ± 0.18a	28.99 ± 0.43b	28.94 ± 0.84b	28.81 ± 0.76b	32.86 ± 0.61d	31.25 ± 0.34c
DPPH (% of inactivation)	63.65 ± 1.92a	67.66 ± 1.28ab	71.71 ± 3.40b	68.95 ± 5.47ab	73.94 ± 5.34b	72.35 ± 3.87b
Tartaric acid (g/L)	2.868 ± 0.037a	3.267 ± 0.148bc	3.380 ± 0.010bc	2.942 ± 0.02a	3.172 ± 0.132ab	3.511 ± 0.576c
Malic acid (g/L)	0.512 ± 0.081c	0.498 ± 0.022bc	0.427 ± 0.011a	0.459 ± 0.016ab	0.465 ± 0.018ab	0.460 ± 0.006ab
Lactic acid (g/L)	0.230 ± 0.008bc	0.238 ± 0.004c	0.214 ± 0.016bc	0.203 ± 0.043ab	0.271 ± 0.034d	0.180 ± 0.006a
Acetic acid (g/L)	0.206 ± 0.002a	0.274 ± 0.032b	0.213 ± 0.001a	0.338 ± 0.012d	0.201 ± 0.001a	0.296 ± 0.016c
Total anthocyanins (mg/L)	22.85 ± 1.00b	21.70 ± 0.77b	21.68 ± 0.11b	17.28 ± 1.25a	22.41 ± 0.98b	22.29 ± 2.31b
Total polyphenols (A_{280})	58.6 ± 0.4a	64.9 ± 0.2b	67.1 ± 0.4c	68.0 ± 0.3d	71.8 ± 0.1f	70.0 ± 0.3e
Total tannins (g/L)	4.567 ± 0.110b	4.185 ± 0.010a	4.963 ± 0.080c	4.967 ± 0.130c	5.136 ± 0.300c	5.536 ± 0.110d
Delphinidin-3-glucoside (%)	4.75 ± 0.14c	3.94 ± 0.04a	3.88 ± 0.30a	4.49 ± 0.37bc	4.45 ± 0.16b	4.37 ± 0.09b
Cyanidin-3-glucoside (%)	19.17 ± 1.02c	18.30 ± 0.11bc	16.49 ± 0.53a	18.16 ± 0.30b	18.48 ± 0.07bc	22.09 ± 1.41d
Petunidin-3-glucoside (%)	9.46 ± 0.98c	6.33 ± 0.36b	4.41 ± 0.56a	8.99 ± 0.12c	4.69 ± 0.58a	5.88 ± 0.58b
Peonidin-3-glucoside (%)	44.95 ± 0.12b	48.36 ± 0.05e	47.41 ± 0.42cd	44.50 ± 0.46a	47.77 ± 0.10d	47.31 ± 0.46c
Malvidin-3-glucoside (%)	21.67 ± 0.29b	23.08 ± 0.39c	27.80 ± 0.75f	23.86 ± 0.10d	24.60 ± 0.77e	20.35 ± 0.46a

Results are expressed as mean values, ($n = 3$) ± standard deviations; identical letters in row indicate no significant differences at $p < 0.05$

* 1 = Zymaflore F15, 2 = RC029-2CxRC039-3C(7)-1C, 3 = RC029-2CxRC039-3C(7)-3A, 4 = RC029-1DxRC039-3C(4)-1A, 5 = RC029-1DxRC039-3C(4)-1C, 6 = RC026-3CxRC039-3C(9)-2B

Table 4 Chemical composition of the wines 12 months from the bottling

Analytic parameters	Strains*					
	1	2	3	4	5	6
pH	3.29 ± 0.02bc	3.29 ± 0.02bc	3.25 ± 0.01a	3.33 ± 0.00d	3.30 ± 0.01c	3.27 ± 0.01b
Total acidity	7.38 ± 0.02a	8.04 ± 0.02c	7.78 ± 0.06b	8.41 ± 0.06d	8.44 ± 0.04d	8.79 ± 0.06e
A_{420}	3.494 ± 0.011b	3.149 ± 0.013a	3.204 ± 0.002a	4.321 ± 0.021d	3.819 ± 0.005c	4.467 ± 0.159e
A_{520}	3.184 ± 0.010b	2.856 ± 0.010a	2.864 ± 0.007a	3.419 ± 0.016d	3.308 ± 0.004c	3.479 ± 0.064e
A_{620}	0.981 ± 0.010c	0.743 ± 0.006a	0.726 ± 0.003a	1.247 ± 0.010e	0.846 ± 0.003b	1.153 ± 0.049d
Colour intensity	7.659 ± 0.027b	6.748 ± 0.029a	6.794 ± 0.009a	8.987 ± 0.038d	7.974 ± 0.002c	9.099 ± 0.271d
Colour hue	1.098 ± 0.003a	1.102 ± 0.001ab	1.119 ± 0.003b	1.264 ± 0.007d	1.154 ± 0.003c	1.284 ± 0.022e
Folin–Ciocalteu index	30.62 ± 0.34a	32.14 ± 0.47b	33.70 ± 0.44c	30.68 ± 0.94a	37.12 ± 0.13d	32.19 ± 1.24b
DPPH (% of inactivation)	65.98 ± 3.71a	65.90 ± 2.73a	70.79 ± 2.17ab	71.09 ± 1.89b	72.88 ± 1.28b	72.69 ± 4.01b
Tartaric acid (g/L)	2.690 ± 0.161a	2.626 ± 0.372a	3.258 ± 0.014b	2.758 ± 0.129a	3.752 ± 0.272c	3.476 ± 0.041b
Malic acid (g/L)	0.309 ± 0.037c	0.156 ± 0.016a	0.154 ± 0.051a	0.268 ± 0.071bc	0.162 ± 0.020a	0.256 ± 0.014b
Lactic acid (g/L)	1.465 ± 0.117e	0.784 ± 0.178a	1.137 ± 0.076cd	0.927 ± 0.064ab	1.248 ± 0.126d	1.059 ± 0.164bc
Acetic acid (g/L)	0.513 ± 0.006b	0.530 ± 0.011b	0.473 ± 0.002a	0.594 ± 0.003c	0.696 ± 0.028e	0.629 ± 0.059d
Total anthocyanins (mg/L)	18.98 ± 1.41c	15.03 ± 0.69a	16.23 ± 1.44ab	14.44 ± 2.41a	18.76 ± 0.65c	18.03 ± 0.28bc
Total polyphenols (A_{280})	55.4 ± 0.2a	61.3 ± 1.1b	62.0 ± 0.2bc	56.3 ± 2.1a	69.5 ± 0.6d	63.7 ± 0.6c
Total tannins (g/L)	3.510 ± 0.020a	4.510 ± 0.170c	4.020 ± 0.170b	3.880 ± 0.030b	3.860 ± 0.100b	4.480 ± 0.070c
Delphinidin-3-glucoside (%)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Cyanidin-3-glucoside (%)	14.77 ± 1.33a	15.86 ± 0.82a	15.52 ± 0.84a	21.36 ± 4.83b	22.45 ± 2.30b	22.88 ± 0.09b
Petunidin-3-glucoside (%)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Peonidin-3-glucoside (%)	67.42 ± 0.92b	65.91 ± 2.12b	60.68 ± 2.94a	74.38 ± 5.83c	77.55 ± 2.30c	77.12 ± 0.13c
Malvidin-3-glucoside (%)	17.80 ± 0.41c	18.22 ± 2.94c	23.80 ± 2.10d	4.26 ± 0.99b	n.d. a	n.d. a

Results are expressed as mean values, ($n = 3$) ± standard deviations; identical letters in row indicate no significant differences at $p < 0.05$; *n.d.* not detected

* 1 = Zymaflore F15, 2 = RC029-2CxRC039-3C(7)-1C, 3 = RC029-2CxRC039-3C(7)-3A, 4 = RC029-1DxRC039-3C(4)-1A, 5 = RC029-1DxRC039-3C(4)-1C, 6 = RC026-3CxRC039-3C(9)-2B

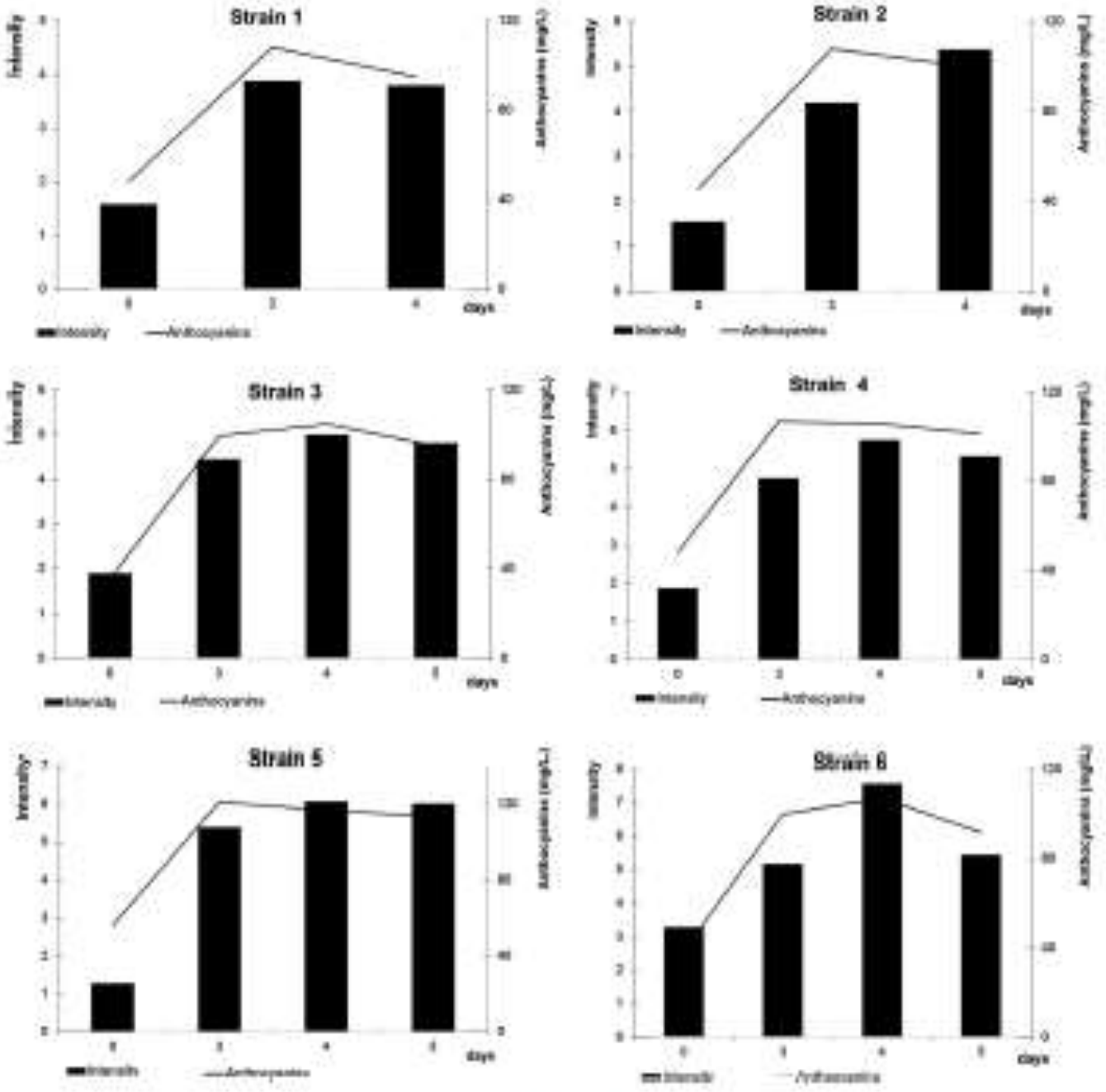

Fig. 1 Anthocyanin content and colour intensity before pressing. Strain number: 1 = Zymaflore F15, 2 = RC029-2CxRC039-3C(7)-1C, 3 = RC029-2CxRC039-3C(7)-3A, 4 = RC029-1DxRC039-3C(4)-1A, 5 = RC029-1DxRC039-3C(4)-1C, 6 = RC026-3CxRC039-3C(8)-2B

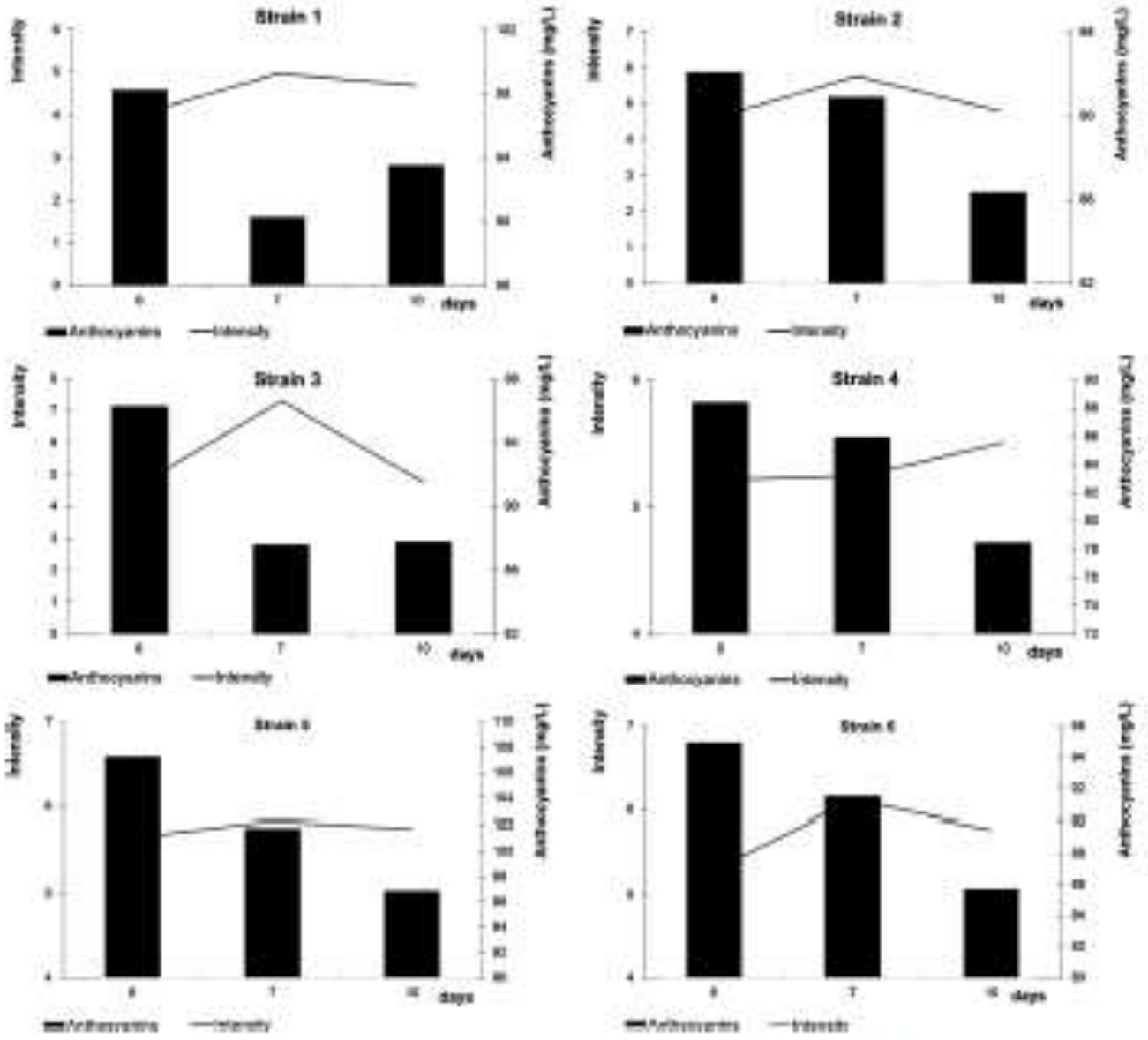

Fig. 2 Anthocyanin content and colour intensity after pressing. Strain number: 1 = Zymaflore F15, 2 = RC029-2CxRC039-3C(7)-1C, 3 = RC029-2CxRC039-3C(7)-3A, 4 = RC029-1DxRC039-3C(4)-1A, 5 = RC029-1DxRC039-3C(4)- 1C, 6 = RC026-3CxRC039-3C(9)-2B

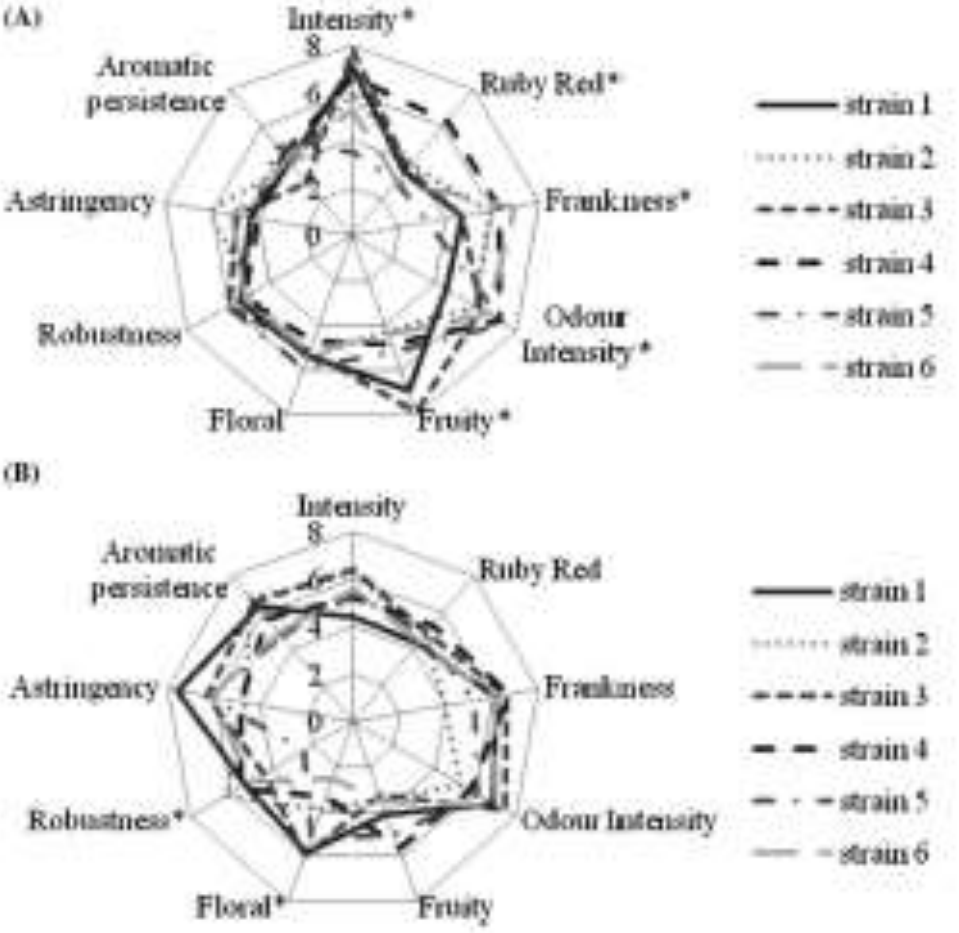

Fig. 3 Sensory analysis of different wines after 8 months of bottling (a), and after 12 months of bottling (b). The asterisk indicates statistically significant differences for $p < 0.05$