Anthocyanin contents and profiles in Sangiovese wines produced with a sequential inoculum of *Starmerella bacillaris* (synonym *Candida zemplinina*) and *Saccharomyces cerevisiae*

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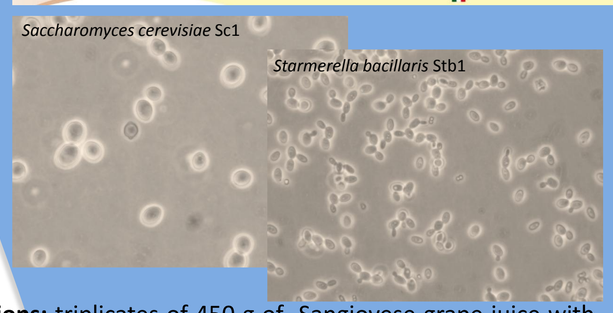

INTRODUCTION. Anthocyanins are the pigments of red grapes and consequently of red wines in which their concentration and composition influence both hue and color stability (1). The anthocyanin profile in Sangiovese grapes, defined as relative abundance of glucosides and acylated anthocyanins, is characterized by a negligible percentage of acylated pigments and high abundance of 3-*O*-glucosides of cyanidin, delphinidin and petunidin (2). However, the concentration of these anthocyanins falls during winemaking with respect to that of methoxylated anthocyanins, such as peonidin 3-O-glucoside and malvidin 3-O-glucoside (3). Indeed, winemaking conditions may play a significant role on the accumulation and partitioning of anthocyanins in wines (4; 5).

Yeast participating in wine fermentation may interact with anthocyanins extracted from grapes. For instance, some strains possess glycosidase activity, including β-glucosidase, which is active on anthocyanin-3-O-glucosides; they produce secondary metabolites such as pyruvate and acethaldehyde that react with grape anthocyanins; they may be responsible of anthocyanin adsorption on cell walls. Among non-Saccharomyces yeasts, *Starmerella bacillaris* (synonym of *Candida zemplinina*) is a yeast species frequently isolated during the early stages of must fermentations and it is known to possess interesting oenological properties: a highly fructophilic character, good tolerance to high glucose concentrations, to ethanol and to low temperatures, a high glycerol and low acetic acid production (6). Moreover, some of the winemaking practices, such as must aeration, are known to directly interact with both phenolic compounds of grapes (7) and fermentative behavior of yeasts (8).

Material and Methods: **Laboratory microvinifications:** triplicates of 450 g of Sangiovese grape juice with its skins and seeds (glucose + fructose, 248 ± 4 g/L; pH, 3.81 ± 0.15; α-aminoacidic N, 128 ± 3 mgN/L) inoculated with specific yeast cultures: **(i)** pure inoculated fermentation, *S. cerevisiae* – Sc1 (10^6 cell/mL), **(ii)** sequential inoculated fermentation, *Starm. bacillaris* – Stb1 (10^6 cell/mL) followed by *S. cerevisiae* – Sc1 (10^6 cell/mL) after 5 days.
Yeast strains, one of each above mentioned species, were previously isolated from spontaneous Brunello di Montalcino wine fermentations and are included in the yeast culture collection of GESAAF, University of Florence, Italy.
The inoculated musts were incubated at 28°C for 14 days. The fermentation progress was evaluated by CO_2 loss, weighting flasks after mixing by gentle rolling one time/day (1 minute) in two conditions: **(i)** open flask (aerated), **(ii)** close flask (non-aerated). The complete Exp plan is reported in table 1. **Chemical analysis**: glucose, fructose, acetate, pyruvate, glycerol and ethanol - HPLC [9]; non-Polymeric and polymeric Anthocyanins HPLC-UV-DAD detector at 520nm [10]. **Statistical analysis**: two-way ANOVA by GraphPad Prism 5 software.

Table 1. Experimental plan and sample denominations

	Pure inoculated fermentation	Sequential inoculated fermentation
Non-Aerated	Sc	Stb/Sc
Aerated	Sc + Air	Stb/Sc + Air

AIM.

The aim of this work was to evaluate the effect of two styles of fermentations on anthocyanin contents and profiles of Sangiovese experimental wines. To this purpose, anthocyanin contents and partitioning, were determined in wines obtained under laboratory conditions by varying both **yeast species** (*Saccharomyces cerevisiae* alone or sequential inoculum of *Starmerella bacillaris* and *S. cerevisiae*) and **aeration conditions** of grape must during fermentation.

RESULTS

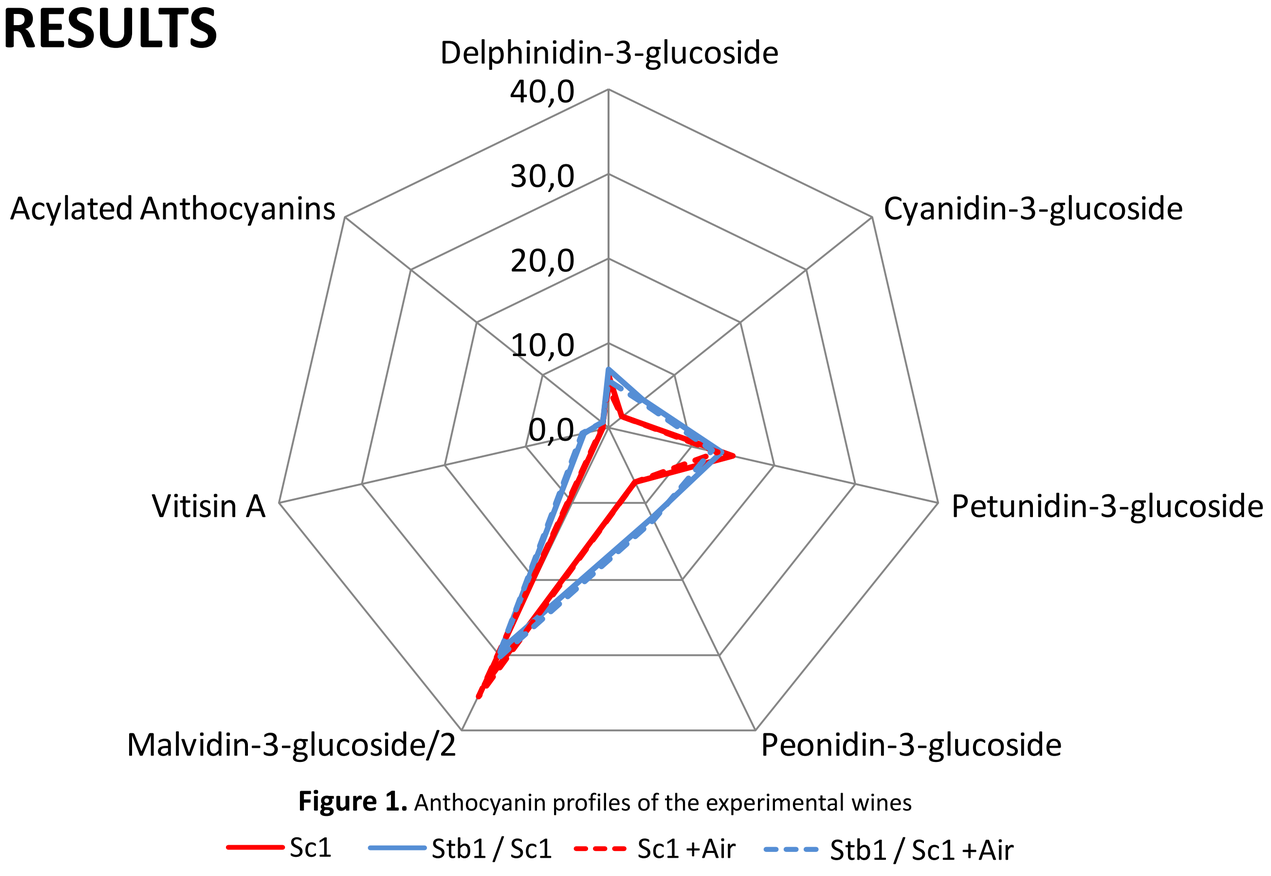

Figure 1. Anthocyanin profiles of the experimental wines

Table 2. Analytical characteristics of Sangiovese experimental wines.

	Experimental wines				Source of variation		
	Sc1	Stb1/Sc1	Sc1+Air	Stb1/Sc+Air	YI	Air	YI xAir
Ethanol (%, v/v)	13.7 ± 0.3	13.4 ± 0.1	13.8 ± 0.0	13.3 ± 0.3	ns	ns	ns
Glycerol (g/L)	10.4 ± 0.0	16.2 ± 0.1	10.1 ± 0.5	15.7 ± 0.1	***	ns	ns
Acetic acid (g/L)	0.11 ± 0.01	0.65 ± 0.01	0.13 ± 0.01	0.52 ± 0.02	***	**	**
Pyruvic acid (mg/L)	10.6 ± 2.1	87.1 ± 10.7	11.6 ± 0.7	100.6 ± 1.4	***	ns	ns
TA ($g_{tart\ ac}$/L)	6.8 ± 0.1	6.5 ± 0.1	6.7 ± 0.1	6.6 ± 0.0	ns	ns	ns
TP (OD_{280})	44.5 ± 0.1	40.4 ± 1.8	40.5 ± 2.4	34.6 ± 3.2	*	*	ns
CI ($OD_{420+520+620}$)	8.2 ± 0.2	8.9 ± 0.4	7.1 ± 0.6	7.6 ± 0.3	ns	*	ns
pH	3.70 ± 0.01	3.67 ± 0.00	3.71 ± 0.01	3.69 ± 0.02	ns	ns	ns

Values are expressed as mean ± standard deviation. *p< 0.05, **p<0.01; ***p<0.001; ns: non-significant. YI = effect of yeast inoculums; Air = effect of aeration; YI x Air = interaction. TA = Total acidity; TP = Total phenols; CI = Color Intensity.

Analytical characteristics of Sangiovese experimental wines (Tab.2)

- The experimental wines produced with **sequential inoculum** of *Starm. bacillaris* Stb1 and *S. cerevisiae* Sc1 were characterized by **higher contents** of **glycerol**, **pyruvic acid** and **acetic acid** and **lower** value of **total phenols** (TP).
- **Must aeration** negatively **influenced** the values of **TP** and **color intensity** (CI)

Table 3. Anthocyanin contents and profiles

	Experimental wines				Source of variation		
	Sc1	Stb1/Sc1	Sc1+Air	Stb1/Sc+Air	YI	Air	YI xAir
non-Pol A (mg/L)	103.2 ± 1.2	99.0 ± 0.7	83.6 ± 2.8	83.7 ± 1.8	ns	***	ns
Pigm Polym (mg/L)	11.0 ± 2.4	11.9 ± 1.2	11.1 ± 0.2	10.4 ± 0.8	ns	ns	ns
A profile (%)							
Dp-3-O-G	6.2 ± 0.2	6.9 ± 0.2	4.7 ± 0.2	5.4 ± 0.4	*	**	ns
Cy-3-O-G	2.0 ± 0.2	5.2 ± 0.1	1.9 ± 0.0	4.7 ± 0.5	***	ns	ns
Pt-3-O-G	15.1 ± 0.1	13.8 ± 0.3	13.1 ± 0.3	12.4 ± 0.4	*	**	ns
Pn-3-O-G	7.2 ± 0.3	11.9 ± 0.2	7.3 ± 0.2	12.2 ± 0.3	***	ns	ns
Mv-3-O-G	68.0 ± 0.2	58.3 ± 0.7	71.0 ± 0.3	61.0 ± 1.6	***	*	ns
Vit A	0.6 ± 0.1	2.9 ± 0.3	1.0 ± 0.1	3.2 ± 0.1	***	*	ns
Acylated A	0.8 ± 0.0	1.0 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	ns	ns	ns

Values are expressed as mean ± standard deviation. *p< 0.05, **p<0.01; ***p<0.001; ns: non-significant. YI = effect of yeast inoculums; Air = effect of aeration; YI x Air = interaction. Non-Pol A = total contents of non-polymeric anthocyanins ; Pigm Polym = Pigmented polymer.

Anthocyanin contents and profiles (Tab.3, Fig. 1)

- **Must aeration** negatively **affects** the **contents** of **non-polymeric anthocyanins**. On the other hand, **polymeric anthocyanins** reached the **same concentration** in all the experimental wines
- **Relative abundances** of anthocyanidin-3-*O*-glucosides and vitisin A were deeply **influenced** by the presence of ***Starm. Bacillaris* Stb1**. In particular, the experimental wines produced with **sequential inoculum** were characterized by a **higher abundance of disubstituted anthocyanins** (Cy and Pt) than in those produced with *S. cerevisiae* Sc1 alone.

CONCLUSIONS

In the present work, a direct **detrimental effect** of **must aeration** was assessed on the accumulation of **non-polymeric anthocyanins** in the experimental wines. Besides, **anthocyanin partitioning** resulted deeply **influenced** by the presence of ***Starm. bacillaris* Stb1** leading to wine with **higher abundance of disubstituted anthocyanins** respect to those produced by *S. cerevisiae* Sc1 alone. This results seemed to be attributable to several species-dependent phenomena of interaction between yeast and anthocyanins.

References. **[1]** He F. et al. (2010) Molecules, 15:9057-9091 **[2]** Mangani S. et al. (2011) Am J Enol Viticult, 62:487-493 **[3]** Arapitsas P. et al. (2012) J Agr Food Chem, 60:10461-10471 **[4]** Sacchi K.L. et al. (2005) Am J Enol Viticult, 56:197-206 **[5]** Suárez-Lepe J. A. & Morata A. (2012) Trends Food Sci Tech, 23:39-50 **[6]** Tofalo R. et al. (2012) Food Microbiol, 29:18-26 **[7]** Castellari M. et al. (2000) Food Chem, 69:61-67 **[8]** Mauricio J. C. (1998) World J Microb Biot, 14:405-410 **[9]** L. Granchi et al. (1998) Food Technol. Biotechnol. 36:313-318 **[10]** OIV (2009) MA-F-AS315-11-ANCYAN 2009. Paris, France.