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Advances in the Molecular Mechanism of Wild Yeast on Color Stability of Red Wine

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Abstract. The color of red wine will change constantly during fermentation and storage, which is related to the yeast introduced. Wild yeast has a strong adaptability to the environment, and effect of which on the color change of red wine is more obvious. In order to explore the molecular mechanism of wild yeast on color change of red wine, this paper summarized the sources and species of wild yeast including influence on production capacity of anthocyanin. The variation of wine color regulated by yeast was related to the genetic material of parent of hybrid strain producing pigment, chromogenic enzyme and storage temperature. Proanthocyanins and yeasts could promote each other. Different yeasts could produce anthocyanins with similar species but greater differences. Therefore, wine color was controlled by various yeasts. Isotopic labeling technology could detect the obvious symbiotic relationship between yeast and anthocyanin, and track accurately and timely the change of wine color. Metabolism of wild yeast and instability of anthocyanin could co-lead to discoloration of wine, however the molecular mechanism of colour fading was not clear nowadays. Therefore, there was an urgent need to screen the wild yeast with the safety and stability to wine color.

INTRODUCTION

Color stability during the aging is one of the most important characteristics of red wines. Wild yeast in grape skin entered the wine through the brewing and was always accompanied by the wine during the whole aging [1-2], which affected the tinctorial stability during the wine aging period as the bottleneck of the stable development of wine industry. Indigenous Saccharomyces Cerevisiae could be isolated from grape leaves, fruits, soils and grape mashes fermented naturally in different wine production areas [1, 3-6]. Therefore, there were a wide range of sources on yeasts.

YEAST

Wine requires yeast to participate in complex biochemical reactions. The fermentation characteristics of yeast are closely related to its own characteristics.

Cheng *et al.* [1] screened out the native Saccharomyces Cerevisiae from the Chardonnay mash fermenting naturally. Yeast could only improve the ability to absorb Ochromycin A at the initial stage of aging, however, yeast cells could reduce the anthocyanin content of wines demanding eliminate toxins in long-term aging, thus yeast strains without effect on anthocyanin should be selected [7]. Therefore, yeast could also cause the instability of wine color, which was related to the type of yeast [7-8]. Medina *et al.*, [2] screened different genera of forty-nine non-

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Saccharomyces yeast strains with effects on anthocyanins from Tannat grapes, which affected significantly color intensity, total anthocyanins, total polyphenol index and hue (p <0.001). Significant variations of six strains, *Metschnikowia pulcherrima*, *Hanseniaspora guilliermondii*, *H. opuntiae*, *H. vineae*, and *H. clermontiae,* were evaluated by the production of anthocyanin-derived pigments compared with *Saccharomyces cerevisiae*. genera *Hanseniaspora* and *Metschnikowia* were reported firstly owe to the origin of malvidin-3-glucoside-4-vinylphenol and malvidin-3-glucoside-4-vinylguaiacol. Bărbulescu *et al.* [3] segregated Saccharomyces Cerevisiae from grape leaves, fruits and soil during the harvest time. Wang *et al.* [9] screened Pichiakluyveri, Hanseniasporauvarum, Saccharomyces Cerevisiae and Cryptococcus magnus in the soil from the natural fermentation of grape juice by agar medium combining with 26S rDNA D1/D2 series analysis. Suranska *et al.* [10] isolated 120 species of native Saccharomyces Cerevisiaes from the grape fruit and natural fermentation of juice by Polymerase Chain Reaction (PCR) fingerprinting and imprinting technique. Bader *et al.* [4] did wine yeasts from different regions of wine grapes. Clemente-Jimenez *et al.* [5] analyzed the population dynamics of the yeasts in six varieties of must from the "Valle del Andarax" area during spontaneous fermentation, identified members of the genera *Candida, Hanseniaspora, Issatchenkia, Metschnikowia, Pichia and Saccharomyces* by PCR-RFLP of the ITS region. Jiang *et al.* [6] separated yeasts from Cabernet Sauvignon grapes, and identified preliminarily four strains of wine yeasts through identification of colony characteristics, mycelial morphology and lysine medium. Legras *et al.*, [8] deduced out that almost all of these flor strains belonged to the same cluster and were diploid, except for a few Spanish strains by the population structure of European flor strains from diversity of microsatellite genotype. Some genes, including *YKL221W/MCH2* and *YKL222C*, were amplified in the genome, with Correlating *ICR1* ncRNA and *FLO11* polymorphisms with population structure of flor yeast, and associating the presence of wild type *ICR1* and a long Flo11p with formation of thin velum in a cluster of Jura strains. The above researches showed that the sources and species of yeasts are very rich.

The difference of wine color was determined by the metabolic discrepancy of disparate yeast strains, and the genetic material of the parent of the hybrid strain producing pigment affected the metabolites of yeast fermentation related to wine color [11], Caridi [12]screened out the yeast strains grown on different lots of the chromogenic medium most suitable for extracting the pigment in the red grape juice of fermentation by an existing low-cost and simple but consistent culturing technique, which provided an effectual method to characterize wine yeasts related to pigment adsorption. Therefore, yeast could regulate the variation of wine color.

Merín *et al.* [13] discussed role of pectinolytic yeasts of Argentine Bonarda grape during spontaneous fermentation, characterized activity of selected yeasts producing extracellular pectinases. 7 genera were identified by partial sequencing of 26S rRNA gene using PCR-DGGE. with *Aureobasidium pullulans* as the most predominant pectinolytic species, followed by *Rhodotorula dairenensis* and *Cryptococcus saitoi*. *R. dairenensis* GM-15 produced pectinases with highly active at grape and vinifications, including cellulase activity, but without β-glucosidase activity at 12 °C and pH 3.5, which encouraged enological properties in low-temperature. Study of Swangkeaw *et al.* [14] showed that intracellular β-glucosidases, which was separated from *Hanseniaspora* sp. BC9 and *Pichia anomala* MDD24, was working during the early stage of alcoholic fermentation (AF). β-Glucosidase from *Hanseniaspora* sp. BC9 could be slightly inhibited by glucose, while β-Glucosidase from *P. anomala* MDD24 could be done by glucose, fructose and sucrose. Otherwise, one isolated from pichia pastoris was doing during the telocinesia of AF. Piemolini-berreto *et al.* [15] extracted some extracellular pectinases from kluyveromyces marxianus, which improved the content of phennolic substance and anthocyanin in Cabernet Sauvignon wine during the period of steep and fermentation, didn't, however, impact the fermenting power of this yeast, which indicated that there were color enzymes in yeasts and effect of color enzymes in disparate yeasts contained were different.

Oenococcus oeni glycosidase and impregnating enzyme had a marked impact on the change of phenolics and ant hocyanins of grape wines [16-17]. Ortega-Heras *et al.* [17] investigated the higher content of phenolic, anthocyanic compounds and color in *Vitis vinifera cv*. Mencía red wine under the effect of the skin contact time, cold pre-fermentative maceration and commercial macerating enzymes. Maceration with dry ice promoted the production of new pigments increasing colour stability and intensity. However, macerated role of enzymes was the richest ones to the wines. Study of Blazquez *et al.* [18] found that the wine with low chromaticity was fermented by Saccharomyces cerevisiae Hansen, the wine with high chromaticity fermented by hybrid strains between Saccharomyces ceresvisiae and Saccharomyces pastorianus, and Saccharomyces pastorianus could extract the lowest content of total anthocyanins and the highest level of anti - SO_2 pigments, which signified that yeast species could affect the color of wine.

Vilela *et al.* [19] confirmed out three yeast strains and a non-yeast strain by fingerprint identification technology, which improved the acidity and color of the wine with high volatile acid. Study of Yacco *et al.* [20] found that prosnthocyanidins could ensure the viable count of yeast by cleaning up reactive oxygen species in cells, and protect

cells. Therefore, the role between proanthocyanidins and yeasts was of mutual promotion, and there was plenty of yeasts in red wine.

Domizio *et al.*, [21] revealed that the main oenological characteristics on pure cultures of 55 yeasts, pertaining to genera *Hanseniaspora*, *Pichia, Saccharomycodes* and *Zygosaccharomyces*revealed, discovered biodiversity per genus. Many of non - *Saccharomyces*strains propagated purity of fermentation, including outcome of ethanol and secondary metabolite, meanwhile augmented production of polysaccharides and modulated the final levels of acetic acid and volatile compounds depending on the yeast species and the *S. cerevisiae*/non-*Saccharomyce*s inoculum ratio. Most of compounds were detrimental to wine quality at high concentrations before reaching taste threshold. Echeverrigaray *et al.* [22] analyzed 50 local red wines in Brazil including 27 fine wines (*V. vinifera*) and 23 table wines (*V. labrusca*). The spoilage yeasts, classified by RFLP-PCR and sequencing of the ITS1-5.8S-ITS2 and D1/D2 26S rDNA loci, in fine wines (11 %) were less popular obviously than that of table wines (70 %). The majority of isolates, *Brettanomyces bruxelliensis*, varied greatly in peculiar smell, with tolerance ethanol (> 10 %) and sulfite (≥ 0.68 mg/L SO_2). so there were harmful yeasts in wine.

Yeasts would not affect the extraction rate of phenolic compounds, and aging could reduce the concentration of phenolic compounds and anthocyanins in wines soaked for 3 days, whereas aging under the high temperature could accelerate the reduction of anthocyanin concentration [23]. Therefore, the dipping and aging temperature would influence the color of wine.

High concentration of freeze-dried yeast could reduce anthocyanin content after short-term contaction with red wine [24]. Legras *et al.* [8] identified low - hybrid genomic regions based on the S288C genomic probe located in the subtelomere region from two genes (MCH2 and YKL222w) of extended sharing pattern isolated from the flor of aging wines, which was the adaptive response of white yeast to aging. Result of Wu *et al.* [25] proved that the bacterial membranes took on diversity, the formation of which needed mutual accomplishment with the white yeast and higher hydrophobicity on the surface of yeast cells by the association of population structure of white yeast with polymorphisms of ICR1 ncRNA and FLO11, as well as the formation of bacterial membranes with wild type of ICR1 and long Flo11p. Therefore, the flor could affect the color of wine [25].

Caridi *et al.* [26] enhanced the genetic characteristics of natural antioxidant and improved the color and stability in red wine through spore cloning selection and hybridization of stable wild Saccharomyces Cerevisiae. Therefore, the wild yeast was more suitable to the environment than the commercial yeast owe to stronger vitality in wine existed long, which supplied extensive research value.

To sum up, the yeast infecting wine quality had dual function of advantage and disadvantage, formed a mutually-beneficial and symbiotic relationship with wine, participated in fermentation and aging of wine, and influenced color change of wine, however, relevant molecular mechanisms need to be studied further. It was urgent to select suitable yeast strains improving the stability of wine color.

Liu *et al.*, [27] screened endophytic C2J6 and botrytis cinerea strains recently, which laid a technical foundation for the screening study of yeast.

ANTHOCYANINS AND OTHER INGREDIENTS RELATED TO COLOUR OF WINE

The color of wine was affected by expression of structural genes related to anthocyanin biosynthesis by mRNA transcriptions and real-time quantitative polymerase chain reaction (Q-PCR) analysis of 11 structural genes in synthetic grape skin [28]. Anthocyanin synthesis coincided with number increase in genes expression in biosynthetic pathway during berry development. Expression of *CHI1*, *PAL*, and *LDOX* had positive accommodation at 2 - 4 weeks after flowering (WAF), negative regulation at 6 WAF - veraison, nevertheless *DFR* was positive at 8 WAF, and subtractive during veraison and ripeness. *F3'5'H*, *GST*, *CHS3*, *UFGT*, and *OMT* were down between 2 WAF and veraison, and up from veraison to maturity. The transcriptional expressions of structural genes demonstrated positive relationship with anthocyanin level from veraison to maturity, including *OMT* transcriptional level and methoxyl - anthocyanins content, as well as *F3'5'H* level and delphinidin anthocyanins content. *F3H2* expression was up, down and up at 2 WAF, 4 WAF to veraison, and veraison to maturity, respectively. *F3'H* capacity was up, down and up again at 2 WAF, 4 WAF, and 6 WAF to maturity, respectively, which was affected positively by concentration of cyanidin anthocyanin from veraison to maturity. Another, anthocyanin had some characteristics, such as smaller molecule, higher solubility, instability, self-agglomeration, co-coloring with copigment or reaction with ethanol, sugar, peptide and tannin [29-30]. González-Manzano *et al.*, [30] confirmed the materiality of phenolic composition and effective copigmentation, classified wine by grape type and winemaking technology by assessing the importance of copigmentation between anthocyanins and flavanols on the colour expression of blended red wine from

Graciano and Tempranillo. Flavanols induced significant variation of colour in red wines, which was related to the concentration and qualitative composition of flavanol, as well as grape and variety. Yacco *et al.*, [20] investigated structure–activity relationships of tannin isolated from red wine under low pressure chromatography by measuring the thermodynamics of interaction between tannins from five Napa Valley wineries throughout fermentation/maceration. This measurement designed the tannin ability to hydrophobically interact with a hydrophobic surface. The tannin activity was related to seed tannins except for skin and pigmented tannins by drive of molecular size, and could reduce the extent of tannin oxidation determined by conversion yield of phloroglucinolysis. Increased convergence role were driven by molecular mass of tannin other than formation or oxidation of pigmented tannin. Different yeasts could produce anthocyanins with the same species but greater differences [15-18], so the color of wine was controlled easily by many types of yeast.

At the beginning stage of aging, phenols in wine converged with each other or participated in complex biochemical reactions, which resulted in variation of structure and content, led to a slow decline in color [31]. Anthocyanidins were combined into macromolecular condensed tannins during the new wine of aging, which caused pigment precipitation [15], acyl chloride of free anthocyanins reaction with polysaccharide and peptide formed a reddish brown or yellow polymer with complex structure and strong stability with phenol, which had something to do with not only precipitation role of protein, polysaccharide and condensed tannin as well as irreversible and steady anthocyanins derivatives, and but also polymer anthocyanins aggregated by the anthocyanins or flavanol including its derivatives [32], which was the result of yeast metabolism.

Storage technology at 4 °C after further fermentation accomplishment in wine isolating residue after fermentation of 4 days was more advantageous to the formation and improvement of most of the monomeric phenols, which was related to the interaction of temperature between free phenols and yeasts. Procyanidine aggregated into by tannins, condensed by catechins and epicatechin, combined into co - anthocyanins with anthocyanin, as well as phenolic acids combined with anthocyanins, which affected the color stability of wine. Therefore, combined action of yeast metabolism and anthocyanin instability led to instability of wine color, but the mechanism needed study further, which was consistent with the dual function of phenols on promoting and inhibiting wine oxidation [33]. After several generations, the biological characteristics of yeast began to degrade and the metabolites would be unstable. The yeast genus *Brettanomyces* in red wine was the only main microorganism converting hydroxycinnamic acid into necessary values, especially 4 - ethylphenol and 4 - ethylguaiacol. When level of 4 - ethylphenol surpassed the sensory threshold, organoleptic characteristics of wine would be influenced or damaged. The *p* - coumaric acid and volatile phenol in wine were concerned with the physicochemical, biochemical and metabolic factors [34], which caused the serious degradation of wine color further. Therefore, yeast metabolism and anthocyanin instability led to wine discoloration together, whereas the molecular mechanism was not clear at present. As mentioned before, wild yeast had stronger suitability to the environment. Therefore, there was an urgent need to screen the wild yeast with safe and stable role to wine color.

ISOTOPE LABELING TECHNIQUE

Gleichenhagen *et al.* [35] labeled ^{13}C on polyphenols of growing plants with $^{13}CO_2$, rather than anthocyanins, in order to generate highly labeled compounds, and analyzed the total content of ^{13}C and individual polyphenols by Isotopic Ratio - MS and HPLC – Iontrap - MS^n. After 34 days, labeling degree of ^{13}C for most polyphenols was higher than 90 atom%, while ^{13}C of total plant material exceeded 88 atom%, which could be used to dissect metabolism and bioavailability of polyphenols. Czank *et al.* [36] synthesized five ^{13}C proanthocyanidin - 3 - glucosides (contained three ^{13}C atoms on the A ring and 2 ^{13}C atoms on the B ring) using carbon isotopes at the position of 6, 8, 10, 3’ and 5’ to investigate the absorption, distribution, metabolism and disturbance status of anthocyanins. Procyanidin - 3 - glucoside was degraded into 6 - hydroxyanthocyanidin - 3 - glucoside by using isotope - ratio mass spectrometry and liquid chromatography - tandem mass spectrometry.

The obvious symbiotic relationship between yeast and host could be found by isotope labeling technology. Nisbet *et al.* [37] used 13C markers and gas chromatography combustion isotope ratio mass spectrometry (GC - C - IRMS) to track changes of yeast and hexose from the degree of ^{13}C enrichment during wine fermentation, and found that hexanol was the only compound derived from grape compounds other than sugars, which could also be applied to other complex systems, such as Maillard reaction, to clarify the contributions of different pathways.

To explore metabolic pathway of grape phenolic regulated by disparate autumn and environmental condition, Chassy *et al.* [38] designed a technique incorporating a stable-isotopic tracer, l - phenyl - $^{13}C_6$ - alanine (Phe^{13}), into Cabernet Sauvignon grape berries *in situ* in the vineyard during the primary ripening or 4 weeks later with high

throughput analytical method depending on LC - DAD - MS/MS to quantify and track the label into phenylalanine metabolites. In consequence, Phe^{13} was labeled in flavonols and anthocyanins, shifted 6 amu of molecular ion, of berries after 9 days. Especially, anthocyanins nearly accounted for the vast majority of label, which was a sign of tight regulation for phenolic biosynthesis at maturity stage. Chassy *et al.* [39] elucidated further the ripening profile of phenolic compound in Cabernet Sauvignon and traced the accumulation level of labeled and unlabeled phenolics during different maturity stage by incorporating in stable - isotope tracer L - phenyl - $^{13}C_6$ - alanine (Phe^{13}), which was consistent with previous research [38]. Concentration of individual anthocyanins, such as malvidin, was climbing abidingly, whereas other verged to plateau or drop during the final development stage owe to accumulation during the maturity. The incorporation continued the extended period after Phe^{13} was under 1 nmol/berry, preventing an accurate assessment of anthocyanin turnover, and implying the presence of substrate in phenolic pathway.

According to the analysis above, the yeast has wide sources and various species. The same yeast, with the dual function of color-protecting and fading, could produce a variety of active products, which was related to the action mechanism of yeast. There was extensive research value. Therefore, it was necessary to carry out the research on the screening, identification, diversity of wild yeast in wine as well as the effect mechanism of wild yeast on the color of wine.

DEVELOPMENT TRENDS IN THE FUTURE

Some technologies, such as molecular fingerprinting, high - throughput sequencing technologies, tracer technology, could provide basic theory guidance for the production of wine including related industries, supplement research contents on wine pigment material by some methods, for example research of screening, identification and spectrum analysis of yeasts producing color, establishment of germplasm repository of wild yeast producing pigment material in wine. In addition, multivariate statistics and principal component analysis were used to establish the correlation between the diversity and abundance of wild yeast communities for different varieties of vintage wine in different producing areas and the major pigment substances, to explore the relationship between wild yeast and wine color as well as the mutative mechanism of color wine during the aging period.

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