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The content of linoleic acid in grape must influences the aromatic effect of branched-chain amino acids addition on red wine

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ABSTRACT

The effect of adding amino acids on wine aroma is largely influenced by nutritional status of grape must. In this study, the effects of linoleic acid (LA) content on the aromatic function of branched-chain amino acids (BCAAs) addition were investigated in alcoholic fermentation of Cabernet Sauvignon wine. The results showed that initial LA content in must significantly influenced the effect of BCAAs addition on volatiles in final wine. Adding BCAAs (140 mg/L of L-leucine, 117 mg/L of L-isoleucine and 118 mg/L of L-valine) in must with low LA content (12 mg/L) promoted the production of most volatiles, including higher alcohols (isobutanol, 2-phenylethanol), fatty acids (hexanoic acid, octanoic acid, decanoic acid) and esters (ethyl acetate, isoamyl acetate, 2-phenethyl acetate and ethyl octanoate), which were well consistent with previous literatures. However, this function disappeared or even became inhibition with increasing LA content in must, especially in 120 mg/L LA must, the total contents of higher alcohol, acetate esters and ethyl esters were 33.9%, 18.1% and 54.2% lower than those in the control without BCAAs addition, respectively. The transcriptional data revealed that several major genes including *GAP1*, *ADH1*, *ATF1*, *ACC1*, *FAS1* and *OLE1* were marked repressed by high LA content. Our data indicated that LA can regulate the expressions of related functional genes to efficiently influence the formations of volatiles in BCAAs supplemented wines. Therefore, it is essential to consider initial content of unsaturated fatty acids (LA) in must when using the strategy that supplying amino acids (BCAAs) to modulate aromatic quality of wines.

1. Introduction

The importance of yeast assimilable nitrogen (YAN) to yeast growth and fermentation metabolism is well known in the wine industry. The modification of composition and amount of YAN can regulate the formation of yeast biomass and fermentation rate and ultimately influence the composition of aroma compounds in wine (Bell & Henschke, 2005; Swiegers, Bartowsky, Henschke, & Pretorius, 2005). In this context, adding nitrogen (ammonium or amino acids) to grape must prior to alcoholic fermentation is a common method to improve fermentation activity and wine aroma quality. Compared to the potential microbiological instability and increased carcinogenic ethyl carbamate caused by ammonium addition, the supplementation of amino acids is an alternative safer approach to improve wine aroma quality. The addition of specific amino acids has targeted aromatic effects on final wine product because these amino acids are the precursors of aromatic compounds formation (López-Rituerto, Avenoza, Busto, & Peregrina, 2010; Torrea et al., 2011). Branched-chain amino acids (BCAAs, including L-valine, L-leucine and L-isoleucine) are important flavour precursors in grape must. Supplementation of BCAAs (leucine and valine) could directly increase the concentrations of isoamyl acetate and isobutanol, indicating that there was a positive relationship between the degradation of BCAAs and the formations of corresponding higher alcohols and esters (Fairbairn, Mckinnon, Musarurwa, Ferreira, & Bauer, 2017). Once added, BCAAs are firstly transported into cells by amino acid permease (encoded by *GAP1*, *BAP2*), which are assimilated by yeast *via* the Ehrlich pathway to biosynthesize higher alcohols with action of aminotransferase (encoded by *BAT1* and *BAT2*),

Abbreviations: LLA, low linoleic acid (12 mg/L); LLA + AA, low linoleic acid with branched-chain amino acids addition; MLA, medium linoleic acid (120 mg/L); MLA + AA, medium linoleic acid with branched-chain amino acids addition; HLA, high linoleic acid (240 mg/L); HLA + AA, high linoleic acid with branched-chain amino acids addition

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decarboxylases (encoded by *PDC1*, *PDC5*, *PDC6*, *ARO10*) and alcohol dehydrogenases (encoded by *ADH1*, *ADH2*, *ADH3*, *ADH4* and *ADH5*) during fermentation (Hazelwood, Daran, van Maris, Pronk, & Dickinson, 2008; López-Rituerto et al., 2010). These alcohols, catalyzed with acetyl-CoA, can further form the corresponding acetate esters with the catalyzation of alcohol acetyltransferase (AATases) encoded by *ATF1* (Swiegers et al., 2005). Our previous study indicated that the positive effect of BCAAs addition on major volatile compounds in wine were largely associated with the increment of amino acid transportation (up-regulation of *GAP*) and yeast population (Liu et al., 2017).

It should be pointed out that the metabolism of amino acids by yeast and their impact on aromatic compounds of wine are largely influenced by nutrition status and fermentation conditions, such as initial YAN content, amino acid composition and fermentation temperature. Adding ammonium salts and/or amino acids to must with rich or deficient nitrogen status could result in different aromatic profiles (González-Marco, Jiménez-Moreno, & Ancín-Azpilicueta, 2010; Torrea et al., 2011), which could be ascribed to the fact that the variation of specific amino acids contents can change the order and efficiency of other amino acids consumed by yeast, and in turn affect the ratio of secondary metabolites produced (Hernández-Orte et al., 2002; Wang et al., 2016). Additionally, fermentation conditions such as temperature variation also have a distinct effect on the uptake and metabolism of amino acids in yeast, and lead to different profiles of volatile compounds (Rollero et al., 2015; Salvadó et al., 2016).

Unsaturated fatty acids (UFAs) represent the major components of the total lipids in grape berries, in which, linoleic acid (C18:2n6, LA) is the most abundant fatty acid in must, its content varied from trace to 280 mg/L dependent on varieties, cultivation and fermentation managements (Duan et al., 2015; Santos et al., 2011; Tumanov et al., 2015). UFAs are essential nutrients for *Saccharomyces cerevisiae* to grow under anaerobic conditions during wine fermentation, which help yeast maintain membrane integrity and function to well adapt to fermentation stresses (Calderbank, Keenan, & Rose, 1985). UFAs can also directly influence the production of volatile compounds (including medium-chain fatty acids and esters) *via* regulating the formation of precursor acyl-CoA and the expression of related genes (Sumby, Grbin, & Jiranek, 2010; Swiegers et al., 2005; Trotter, 2001; Yoshioka & Hashimoto, 1983). Changing UFAs contents in must can effectively influence yeast growth and modify the formation of yeast-derived volatile compounds (Sumby et al., 2010). Due to the importance of nitrogen and fatty acids in wine fermentation, several researchers have investigated the synergetic impact of nitrogen and lipids/fatty acids on the formation of aroma compounds in wines. Rollero et al. (2015) found a positive relationship between nitrogen (ammonium and amino acids) and phytosterol content for the production of isobutyl alcohol and isoamyl alcohol, while González-Marco et al. (2010) observed adding yeast cell autolysate (including long-chain saturated and unsaturated fatty acids and assimilable amino acids) had no significant difference of most aroma compounds. The inconsistent results could be due to the different composition of added lipids/fatty acids and amino acids pool.

Adding amino acid into must is a popular technique in modern winemaking to improve aroma quality of wine products, but this effect is still lack in-depth understood, especially the synergetic impact of amino acid additions and other nutritional composition. To enrich our understanding the impact of amino acid addition on aromatic quality of wine, in this work, the effects of LA contents variation in grape must on the fermentation performance and volatile formation were investigated in Cabernet Sauvignon wine, which was initially supplemented with BCAAs (including L-valine, L-leucine and L-isoleucine). The obtained results indicated that the effects of adding BCAAs on volatile formation were largely influenced by LA content in grape must. The determination of transcriptional profiles revealed that the distinct effect was mainly associated with the variation of related functional gene expressions.

Table 1
Composition of grape must with different treatments (low linoleic acid without (LLA) and with branched-chain amino acids (LLA + AA), medium linoleic acid without (MLA) and with branched-chain amino acids (MLA + AA), high linoleic acid without (HLA) and with branched-chain amino acids (HLA + AA)).

Parameter	LLA	LLA + AA	MLA	MLA + AA	HLA	HLA + AA
Sugar (g/L)	212	212	212	212	212	212
pH	3.3	3.3	3.3	3.3	3.3	3.3
Valine (mg/L)	26.2	117.8	26.2	117.8	26.2	117.8
Leucine (mg/L)	14.5	140	14.5	140	14.5	140
Isoleucine (mg/L)	13.1	117	13.1	117	13.1	117
YAN (mg N/L)	163	198	163	198	163	198
Linoleic acid (mg/L)	12	12	120	120	240	240

YAN, yeast assimilable nitrogen.

2. Materials and methods

2.1. Strain and culture medium

Fermentations were carried out in grape must from *Vitis vinifera* Cabernet Sauvignon, with the commercial wine yeast *S. cerevisiae* var. *bayanus* strain EC1118 (Lallemand, Blagnac, France). This commercial wine yeast is used worldwide for both red and white winemaking, and is considered a fast and robust fermenting strain, with a neutral contribution to the wine aroma (Molina, Swiegers, Varela, Pretorius, & Agosin, 2007; Pinu, Edwards, Gardner, & Villas-Boas, 2014). The grapes were collected in Changli wine region of China. Sulfur dioxide (60 mg/L) was added into the must after the de-stemming and crushing. The initial sugar concentration was 212 g/L, and the YAN, 163 mg/L, which could meet the requirement of yeast normal growth (Bell & Henschke, 2005) (Table 1). The initial concentration of linoleic acid in must was 12 mg/L (LLA). Two levels of additional linoleic acid (increased to 120 mg/L, MLA, and 240 mg/L, HLA) were designed according to our preliminary work (Data not shown). Branched-chain amino acids, L-leucine, L-isoleucine and L-valine, were added to 140 mg/L, 117 mg/L and 118 mg/L, respectively, based on preliminary work in our laboratory (Liu et al., 2017; Wang et al., 2016). The treatments were named LLA + AA, MLA + AA and HLA + AA, respectively. The final concentration of BCAAs and linoleic acid was within the common range in grape must (Duan et al., 2015; Santos et al., 2011; Tumanov et al., 2015).

2.2. Fermentation conditions and sampling

Saccharomyces cerevisiae was inoculated into 500 mL yeast extract peptone dextrose (YPD) medium (20 g/L glucose, 10 g/L peptone, 5 g/L yeast extract) at 30 °C with shaking (150 rpm) overnight. After harvested and washed with sterile water twice, initial viable population of 10^6 CFU/mL cells were inoculated into grape must. The fermentations were conducted in 500 mL flasks sealed with a fermentation lock and a puncture needle for sampling. The flasks were filled with 350 mL must and maintained at 25 °C without shaking. All treatments were in triplicate.

The progress of fermentation was monitored by cell density (optical density at 600 nm, OD_{600nm}) and the consumption of sugar. Dry cell weight (DCW) was calculated from OD_{600nm} values using the following equation: DCW (g/L) = 0.3 × OD_{600nm}, which was obtained in our preliminary work based on the methods of Molina et al. (2007). Samples of 30 mL of culture medium were taken in mid-exponential, early-stationary and late-stationary growth phase and centrifuged for 10 min at 13,800 ×*g*. Supernatant samples were stored at −20 °C for the analysis of amino acids, fatty acids and volatile compounds. Cells were harvested and stored at −80 °C for RNA isolation.