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Evaluating the influence of maceration practices on biogenic amine formation in wine

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ABSTRACT

Biogenic amines are formed during winemaking from precursor amino acids, mainly by lactic acid bacteria during malolactic fermentation (MLF). Various factors can influence the amino acid content of the grape must and wine; including contact with the grape skins before, during and after alcoholic fermentation. The quantity and composition of amino acids in the must can potentially dictate the subsequent formation of biogenic amines. In this study we investigate the influence of compounds extracted from the grape skins by different maceration practices applied during winemaking on the formation of biogenic amines. Wines were made on small scale with two red grape cultivars. Treatments consisted of free-run juice (no skin contact), skin contact during alcoholic fermentation, cold maceration and extended maceration; followed by MLF in all treatments. Our results show that higher levels of precursor amino acids and biogenic amines were detected in the absence of skin contact, extended maceration and to a lesser extent in conventional maceration. Cold maceration before fermentation initially increased the extraction of amino acids and formation of biogenic amines, but resulted in the lowest concentrations of these harmful compounds in the final wines. Cold maceration therefore appears to have a protective effect against biogenic amine accumulation during MLF.

1. Introduction

The maceration of grape skins during vinification promotes extraction of grape components such as phenolic compounds, proteins, amino acids and polysaccharides. Grape skin maceration practices commonly applied during red wine production include cold maceration (cold soaking before alcoholic fermentation); alcoholic fermentation in contact with grape skins (conventional maceration) and extended maceration after alcoholic fermentation. Pectolytic enzymes are often added to grape must to increase the yield of juice, clarify the must, extract compounds from the grape skins and to facilitate pressing and filtration. Carbonic maceration and thermovinification are less conventional grape skin extraction methods that can be applied during winemaking (Boulton, Singleton, Bisson, & Kunkee, 1996, p. 42).

Biogenic amines are organic nitrogenous compounds formed by the metabolisms of living organisms (microorganisms, plants and animals) from amino acid precursors. In fermented foodstuffs, such as wine, the non-volatile biogenic amines (histamine, putrescine, cadaverine, spermine, spermidine, agmatine, tyramine, and tryptamine) and phenylethylamine (a volatile amine) are formed by microbial decarboxylation of the corresponding amino acids (Ten Brink, Damink, Joosten, & Huis in't Veld, 1990). In wine, biogenic amines can originate from the grape berries or can be produced during fermentation, ageing or storage if conditions persist that favour the growth of decarboxylase positive microorganisms and the activity of the relevant decarboxylase enzymes (Spano et al., 2010). The storage of grapes prior to crushing under non-sterile conditions could also influence biogenic amine concentrations (Cecchini & Morassut, 2010). Lactic acid bacteria (LAB) are the main microorganisms responsible for biogenic amine production in wine (Moreno-Arribas, Smit, & Du Toit, 2010; Smit, Du Toit, & Du Toit, 2008).

The levels of biogenic amines produced in wine greatly depend on the abundance of amino acid precursors in the medium. Generally, biogenic amine production will increase with increased availability of free amino acids. Amino acid content in grape must,

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and subsequently in wine, may be influenced by vinification methods, grape variety, geographical region, vintage and vine nutrition (Herbert, Cabrita, Ratola, Laureano, & Alves, 2005; Lonvaud-Funel & Joyeux, 1994; Moreno-Arribas, Torlois, Joyeux, Bertrand, & Lonvaud-Funel, 2000; Soufleros, Barrios, & Bertrand, 1998). Grape varieties with higher levels of amino acids have been found to yield the higher final concentrations of biogenic amines (Herbert et al., 2005). The distribution of amino acids between the skin, pulp and seeds within the grape berry is possibly influenced by vineyard practices, terroir and grape variety. The amino acid content in the skins of Cabernet Sauvignon berries in France was found to represent 53% of the total amino acids in the grape berry, with 40% occurring in the pulp and 7.8% in the seeds (Miele, Carnonneau, & Bouard, 2000). In a similar study, Australian Cabernet Sauvignon berries were found to contain only 15%–23% of the total grape amino acids in the grape skins, while approximately 77% was located in the pulp and 8.5% in the seeds (Stines et al., 2000). Thus, it is possible that a large proportion of amino acids may be located in the grape skins and that prolonged contact between must or wine and the skins could increase their extraction into the wine.

Amino acids are utilised as a source of nitrogen for growth and metabolism during wine fermentations by yeasts and LAB. Therefore, the concentrations of amino acids in general and potential precursors of biogenic amines in particular, may change considerably during wine fermentations. Changes in amino acid content may be due to the actions of yeasts, including the secretion of excess amino acids by yeasts and the release of amino acids by yeast autolysis (Soufleros et al., 1998). Amino acid composition and concentration can also be changed by LAB. As a result of maceration practices amino acids can be released from grape skins, extracted from grape skin derived components, or from yeast autolysates by the action of proteolytic and peptolytic enzymes of LAB (Leitão, Teixeira, Barreto Crespo, & San Romão, 2000; Manca de Nadra, Farais, Moreno-Arribas, Pueyo, & Polo, 1999). Commercial pectolytic enzyme preparations may contain proteolytic activity which can lead to the release of amino acids from peptides and proteins.

Conflicting results regarding the correlation between grape skin maceration practices and the levels of biogenic amines in wines are reported in literature. Soleas, Carey, and Goldberg (1999) found no correlation between the skin contact time and concentration of biogenic amines. However, other authors found the duration of skin maceration to be an important variable affecting biogenic amine content in wine, with longer skin contact time potentially favouring increased production of biogenic amines (Bauza, Blaise, Daumas, & Cabanis, 1995; Martín-Álvarez, Marcobal, Polo, & Moreno-Arribas, 2006). With regards to the influence of commercial enzymes on biogenic amines, Martín-Álvarez et al. (2006) concluded that pectolytic enzymes added to the grapes did not promote biogenic amine accumulation in their wine. In contrast, the concentration of histamine was found to increase upon addition of a commercial pectolytic enzyme preparation to elderberry fruit wine (Pogorzelski, 1992). Other winemaking practices such as hot maceration (Pogorzelski, 1992) and reductive fermentation conditions induced during the Ganimede vinification method, also resulted in higher biogenic amine concentrations (Ancín-Azpilicueta, González-Marco, & Jiménez-Moreno, 2010).

The analysis of biogenic amines is of importance to the wine industry because of the toxicity implications of these compounds to humans (Ten Brink et al., 1990), as well as the potential to apply biogenic amines as indicators of spoilage or authenticity (Hajós, Sass-Kiss, Szerdahelye, & Bardocz, 2000). In this study we quantified seven oenologically important biogenic amines (histamine, tyramine, putrescine, spermidine, cadaverine, phenylethylamine and tryptamine) and their respective precursor amino acids (histidine, tyrosine, arginine, ornithine, lysine, phenylalanine and tryptophan) in wines inoculated with LAB microflora containing decarboxylase enzymes. This was done to ascertain the effect of five different grape skin maceration treatments on the production of oenologically relevant biogenic amines. To our knowledge, no systematic study of the influence of different maceration practices applied during winemaking on precursor amino acids and subsequent biogenic amine formation has been published. This study thus aims to provide an investigation of the influence of compounds extracted from the grape skins during different maceration practices, specifically amino acids and phenolic compounds, on the subsequent biogenic amine production by LAB.

2. Material and methods

2.1. Microorganisms, culture media and growth conditions

LAB previously isolated from South African brandy base wines and identified as histidine-, tyrosine- and/or ornithine decarboxylase positive (Downing, 2003) were inoculated into the grape must to simulate a natural microflora with the potential to produce biogenic amines. This was done not to replace the existing natural microflora, but to ensure the presence of decarboxylase positive LAB in the grape must that could contribute to biogenic amine production. The presence of decarboxylase genes were verified in the inoculated strains by the multiplex polymerase chain reaction (PCR) assay described by Marcobal, De Las Rivas, Moreno-Arribas, and Muñoz (2005). The strains *Lactobacillus hilgardii* B74 (histidine and ornithine decarboxylase positive), *L. hilgardii* M59 (histidine and ornithine decarboxylase positive) and *Lactobacillus brevis* M58 (tyrosine and ornithine decarboxylase positive) were used. Prior to inoculation into the grape must, the LAB strains were precultured for 36 h in De Man, Rogosa and Sharpe (MRS) broth (De Man, Rogosa, & Sharpe, 1960), followed by 48 h in a MRS-based adaptation medium as described by Lerm, Engelbrecht, and Du Toit (2011). The described adaptation medium was additionally supplemented (during preparation, together with other medium components) with 0.1 g per 100 mL of medium of each amino acid precursor (L-ornithine, L-tyrosine and L-histidine) (Bover-Cid & Holzapfel, 1999) and 0.005 g per 100 mL of medium of pyridoxal-5′-phosphate, the cofactor required for decarboxylase activity (Smit et al., 2008). This was done to facilitate adaptation of LAB to wine conditions such as low pH, high sugar and the presence of ethanol, and to induce decarboxylase enzyme activity. The three LAB strains were pre-cultured separately to a culture density of 10^9 CFU per mL of culture medium. Equal volumes of the three pre-cultures were combined into a single culture, from which wines were inoculated to a final density of 10^6 CFU per mL of wine. The initial survival of the "simulated natural microflora" was confirmed two days after inoculation.

Saccharomyces cerevisiae strain NT202 (Anchor Yeast, Cape Town, South Africa) was rehydrated according to the manufacturer's instructions and inoculated into all treatments at 0.3 g/L to perform alcoholic fermentation.

2.2. Vinification and grape skin maceration treatments

The experimental procedure was repeated with Cabernet Sauvignon and Shiraz grapes; from the Paarl region, South Africa. After grapes were destemmed and crushed, the skins and free-run juice were separated, homogenised and equally divided between treatments. Five litres of free-run juice was used for treatment 1 (control treatment), which did not receive any contact with grape skins. Equal amounts of free-run juice and grape skins were assigned to each of treatments 2–5. Treatment 2 was subjected to cold