LWT - Food Science and Technology 87 (2018) 488–497

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Combined effect of temperature and ammonium addition on fermentation profile and volatile aroma composition of Torrontés Riojano wines

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ARTICLE INFO

Article history:
Received 15 March 2017
Received in revised form
6 September 2017
Accepted 15 September 2017
Available online 20 September 2017

Keywords:
Torrontés Riojano
Fermentation temperature
Ammonium nutrition
Aroma compounds
Terpenes
Yeast
Wine

Chemical compounds studied in this article:
Citronellol (PubChem CID: 8842)
Hotrienol (PubChem CID: 5366264)
Linalool (PubChem CID: 6549)
Isoamyl acetate (PubChem CID: 31276)
Ethyl octanoate (PubChem CID: 7799)
Ethyl decanoate (PubChem CID: 8048)

ABSTRACT

The effects of process temperature and ammonium supplementation on fermentation profile, volatile aroma composition and wine sensory characteristics were evaluated in Torrontés Riojano (Vitis vinifera L.) wines from Mendoza (Argentina). To do so, musts were fermented at two different temperatures (14 y 22 °C), with and without addition of 200 mg/L of yeast assimilable nitrogen, using ammonium salt. The fermentation profile was obtained by daily monitoring weight loss and counting viable yeasts. HS-SPME-GC-MS procedure was applied in order to determine the aromatic profile of wines. Two-way ANOVA and PCA analysis revealed that the treatment fermented at low temperature and with nutrition (14AN) showed the maximal concentration of terpenes and lower levels of pH and volatile acidity, obtaining a better quality wines. However, the treatment fermented at higher temperature and with nutrition (22AN) was identified by the sensory panel as the most aromatic wine, finding notes of pineapple and banana related with its greater amounts of higher alcohol acetates. Low temperature displayed slow fermentations and the nitrogen salts were hardly consumed by yeast in this process condition. Ammonium addition and high temperature increased the maximal fermentation rate producing shorter fermentation times and greater amounts of higher alcohols acetates.

1. Introduction

Torrontés Riojano (*Vitis vinifera* L.) is a widely cultivated Argentinean white variety and has recently been named as native grape resulted of a natural crossing that took place in this country between Moscatel de Alejandría and Listan Prieto varieties (Agüero, Rodríguez, Martínez, Dangl, & Meredith, 2003; Lacoste, Yuri, Aranda, Castro, & Quinteros, 2010). It is characterised by being an aromatic grape with a high terpenoid content based on its relationship with Muscat grapes (Belancic & Agosin, 2009). The predominant free monoterpenes found in this variety are linalool, geraniol, nerol, α-terpineol, β-citronellol, hotrienol and limonene (Romano, Trebes, & Barbeito, 2011), contributing to their wines with honey, rose and azahar notes.

Terpenes are typical varietal aroma compounds present in Muscat grapes in free volatile forms and bound with sugar as glycosides. Free terpenes have low aroma thresholds so they have a high impact on "floral" character of wines (Bayonove, Baumes, Crouzet, & Günata, 2003; Marais, 1983). Their concentrations in grapes are influenced by several factors like maturation degree, soil conditions, viticulture practices and grape cultivar (Belancic & Agosin, 2009).

Additionally there are other volatile compounds formed during yeast fermentation like higher alcohols, medium chain fatty acids

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https://doi.org/10.1016/j.lwt.2017.09.020
0023-6438/

and their corresponding esters, which also play an important role in the overall aroma of the young white wine. All of these compounds are affected by several fermentation factors like nutrition and fermentation temperature (Molina, Swiegers, Varela, Pretorius, & Agosin, 2007; Mouret et al., 2014).

Alcoholic fermentation at low temperature (12–20 °C) is nowadays a common practice in white winemaking, aimed to improve aromatic profiles by a high formation and retention of aromas. Furthermore, low temperature can improve the quality of wine in terms of volatile and total acidity, pH, alcohol and glycerol production (Torija, Rozès, et al., 2003). However, low temperature has a strong influence on fermentation kinetics by increasing the probabilities of sluggish or even stuck fermentations, due to the negative effect on yeast growth and to the diminution on fermentation rate (Bisson, 1999). On the other hand, low temperature increased the unsaturated degree of yeast membrane fatty acids making yeast more ethanol tolerant (Torija, 2002).

Additions of ammonium salts to musts are routinely used by winemakers in order to reduce the risk of problematic fermentations. It is well known that these salts and α-amino acids are the main sources of yeast assimilable nitrogen (YAN), which have the capacity to improve biomass synthesis, increase the fermentation rate, and prevent H_2S production (Bell & Henschke, 2005). Nitrogen supplementation also regulates the formation of many volatile and non-volatile compounds that can contribute to the improvement of the final flavour of the wines (Carrau et al., 2008; Vilanova, Siebert, Varela, Pretorius, & Henschke, 2012).

There are several researches about the combined effects of temperature and nitrogen nutrition on aromatic composition of wine, but most of them studied above volatile compounds released by yeast. They affirm that nitrogen addition is the most influence factor on the production of desirable fermentative aromas and the effect of temperature is linking to the volatilization or retention effect on the concentration of them (Mouret et al., 2014; Rollero et al., 2015).

Despite its economic and cultural importance to Argentina, Torrontés Riojano cultivar has been subject of only few studies focused on different aspects like grape characterisation (Agüero et al., 2003), the influence of the origin region (Romano et al., 2011) and its genealogy (Lacoste et al., 2010). However, the impact of fermentation factors on its aroma has been poorly studied (Pérez, Díaz-Quirós, Assof, Sari, & Casassa, 2014). Thus, the aim of our work was to evaluate the individual and interaction effects of two relevant fermentation factors (temperature and ammonium nutrition) over the aroma production and fermentation performance on Torrontés Riojano wines.

2. Materials and methods

2.1. Standards and reagents

All standards such as anisole [100-66-3], linalool [78-70-6], citronellol [106-22-9], geraniol [106-24-1], nerol [106-25-2], limonene [5989-27-5], α-terpineol [98-55-5], α-terpinolene [586-62-9], β-ocimene [13877-91-3], 1-hexanol [11-27-3], 2-ethyl-1-hexanol [104-76-7], decanoic acid [334-48-5], octanoic acid [124-07-2], 2-phenylethanol [60-12-8], isoamyl alcohol [123-51-3], ethyl acetate [141-78-6], hexyl acetate [142-92-7], 2-phenylethyl acetate [103-45-7], isoamyl acetate [123-92-2], ethyl butanoate [105-54-4], ethyl hexanoate [123-66-0], ethyl octanoate [106-32-1], ethyl decanoate [110-38-3], *N*-acetyl-L-cysteine [616-91-1], *o*-phthaldialdehyde [643-79-8], L-isoleucine [73-32-5], were purchased from Sigma-Aldrich (St. Louis, MO). Lead acetate [546-67-8] and antifoaming agent were supplied by Poyén Nutun (Mendoza, Argentina). Activated carbon, sodium chloride, soluble starch and sodium borate decahydrate were obtained from Alkemit (Mendoza, Argentina), potassium hydroxide, boric acid, sodium hydroxide, sulfuric acid, phenolphthalein, and absolute ethanol were supplied by Biopack (Buenos Aires, Argentina) potassium metabisulphite was from Juan Messina S.A. (Mendoza, Argentina), Fehling Causse Bonnans reagent, bromothymol blue and methylene blue were from Mag S.R.L. (Mendoza, Argentina) and iodine solution was obteined from Anedra (Buenos Aires, Argentina). All chemicals used were of analytical grade. Ammonia enzymatic kit was supplied by *R*-Biopharm (Boehringer Mannheim, Darmstadt, Germany).

2.2. Yeast strain

Lalvin EC1118® *Saccharomyces cerevisiae* var. *bayanus*, an active dry yeast provided by Lallemand (Montreal, Canada), was employed throughout this study. Since, we wanted to remove the effect of the strain, this one was chosen based on its neutral effects on aroma, its low nitrogen demand, and its ability to ferment at low temperature. The commercial yeast was rehydrated according to the manufacturer's instructions and was added to musts at 20 g hL^{-1}.

2.3. Plant material and fermentation conditions

The experiment was performed during 2014 season in a Torrontés Riojano vineyard located at INTA Experimental Station, Mendoza, Argentina (68° 51′ W and 33° 00′ S). The grapes were manually harvested at 22 °Brix, transported immediately to the winery, destemmed and pressed. The juice obtained was settled at 4 °C for 24 h and then was decanted. The concentration of must sugar was 218.2 g L^{-1} and the initial YAN concentration was 114.1 mg L^{-1}. In order to test the effects of temperature and nutrition on aroma concentration and fermentation performance, grape must was divided into 4 treatments involving a factorial combination of different amounts of YAN (with and without addition of ammonium salt) and two fermentation temperatures (14 °C and 22 °C). Fermenters were inoculated with an initial viable population of approximately 10^6 cell mL^{-1} with active dry yeast. Fermentations were carried out in 5 L glass bottles filled with 4 L of must. Fermentation performances were followed by daily measuring the weight loss expressed in grams per Litre. To determine the maximum fermentation rate (g L^{-1} day^{-1}) of each treatment, a fermentation curve was fitted by the weight loss (g L^{-1}) measurements versus time, applying the reparametrized Gompertz equation proposed by Zwietering, Jongenburger, Rombouts, and Van't Riet (1990). In the middle point of fermentation (1.060 mg mL^{-1}), two of the flasks were supplemented with diammonium phosphate (DAP) to increase YAN concentration up to 200 mg N L^{-1} (14AN and 22AN) whereas, as a control, no ammonium salt was added to the others two treatments (14WN and 22WN). The treatments were performed in triplicate. At the end of fermentation (fermentation sugar < 1.8 g L^{-1}), wines were cold settled for two weeks. After that, wines were racked, filtered, sulphited (20 mg L^{-1}), and bottled. Some bottles were stored at 8 °C until sensorial analysis, and others were frozen at −4 °C until GC-MS analyses.

2.4. General analytical parameters

Wine parameters such as sugar, alcohol, pH, volatile acidity and titratable acidity were determined according to the international methods provided by the *Office International de la Vigne et du Vin* (OIV., 2013).